BOVINE TUBERCULOSIS IN THE BRUSHTAIL POSSUM (*TRICHOSURUS VULPECULA*)

BEHAVIOUR AND DEVELPOMENT OF AN AEROSOL VACCINATOR

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ABSTRACT

The Australian brushtail possum (*Trichosurus vulpecula*) is a wildlife reservoir of tuberculosis (*Mycobacterium bovis*) in New Zealand. The disease is endemic over one third of the country. Possum control operations have reduced the prevalence of disease in livestock but have not fully controlled infection in wildlife or geographic spread of the disease. The disease is transmitted to livestock when they investigate the unusual behaviour of terminally ill possums. Reduction of disease incidence in possums through vaccination with bacille Calmette-Guerin (BCG) has shown promise both in pen trials and field studies. Integration of vaccination into existing control programmes may reduce transmission of tuberculosis among possums, and from possums to livestock.

There are two parts to this thesis. Part one is a longitudinal, behavioural study of tuberculous and non-tuberculous wild possums. Part two is a description of an aerosol delivery device (aerosol vaccinator) designed to administer aerosolised BCG vaccine to possums in the wild, and a record of its progressive development.

The aim of part one was to identify aspects of behaviour of tuberculous possums that may influence disease transmission to livestock. Twenty two tuberculous and eight healthy possums were observed. Possums were radio tracked weekly and live trapped at bimonthly intervals on a 56 hectare site in the Wairarapa, New Zealand.

Generally possums remained within their activity range apart from infrequent long distance forays. Possums were weak, lethargic and uncoordinated during the terminal stages of disease which lasted for one to three weeks. Only three possums made long forays when terminally ill with tuberculosis.

The carcasses of 17 tuberculous possums were recovered of which 15 were in dense scrub or on long grass under scrub and two were on pasture. Of these 17 carcasses, 14 were within or near (<200m) to their activity range.

Most tuberculous possums died in their activity range and in scrub. These possums represent little risk of infection to livestock, but a risk to other wildlife. However, the small number of tuberculous possums that died on pasture present an important risk to livestock. Interactions between diseased and healthy possums during long distance forays may cause considerable geographical spread of tuberculosis.

Part two, the development of an aerosol vaccinator, consisted of pen and field trials. The aim of pen trials was to evaluate the willingness of possums to investigate novel objects and the influence of social hierarchy on this investigative behaviour. It also allowed refinements to aerosol vaccinator design.

Four captive colonies were used. In each colony most possums (80%) showed minimal neophobia and would actively investigate novel objects. A small proportion (20%) would not approach a novel object. A loose social hierarchy existed with one dominant animal and a changeable middle order. In two of the four colonies, there was one possum clearly at the bottom of the social order. Social hierarchy did not affect the proportion of a colony which could investigate a novel object or vaccinator. However it did effect the order in which individual possums would investigate.

The aim of the field trials was to evaluate the efficacy of an aerosol vaccinator with possums in the wild. During five field trials, the proportion of the possum population marked with dye from the device steadily increased and ranged from 0%–34%. Trials were conducted over eight months and during this period a total of 56% of the study population was marked with dye. Some possums would repeatedly use a vaccinator.

These results justify further research into aerosol vaccination of wild possums with BCG. Three key avenues for future research include determining the proportion of a possum population which will use the device, developing an aerosol container suitable for dispensing BCG vaccine and determining whether the combination of vaccinator and aerosol vaccine elicits a protective immune response.

The aerosol vaccinator may be use to deliver aerosolised materials other than BCG to possums. It may also be altered to suit use by other species.

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LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

For over one hundred years New Zealand has struggled to control the problem of tuberculosis in domestic livestock. This situation has been further complicated by the introduction of the Australian brushtail possum (*Trichosurus vulpecula*).

Tuberculosis caused by *Mycobacterium bovis* is assumed to have been introduced to New Zealand during cattle importation and was considered a serious public health problem during the nineteenth and early twentieth century. In recent years it has also been identified as a potential barrier to New Zealand's large export trade in meat and dairy products (Animal Health Board, 2000). The organism *M. bovis* is a bacterial pathogen which infects cattle primarily via the respiratory tract (Corner *et al.*, 1990). Tuberculosis infection is controlled primarily by the body's cell mediated immune system (Bloom, 1994).

The possum was introduced to New Zealand in the 1850s to start a fur trade (Pracy, 1974) and in the following years it spread rapidly throughout the country. Today it is considered a noxious pest with an estimated population at around 1990 of 70 million (Cowan and Bayliss, 1998). This problem was compounded by the discovery that, in New Zealand, the possum acts as a wildlife reservoir for tuberculosis and is responsible for a major part of the spread of this disease amongst cattle (Ekdahl *et al.*, 1970). Extensive efforts are made to control the possum population. These are based primarily on poisons and trapping, and conducted continuously at great expense.

Aerosol vaccination as a means of protecting animals against a range of diseases has been researched in a range of species, primarily poultry (Jadin, 1980) and pigs (Popa *et al.*, 1982), (Newall and Hewinson, 1995). Aerosol vaccination of possums with bacille Calmette-Guerin (BCG) vaccine has been shown to reduce the effects of infection with tuberculosis (Aldwell *et al.*, 1995).

There is potential to use BCG as an aerosol vaccine to protect wild possum populations against tuberculosis. This may be used as an alternative to, or in conjunction with current methods for the control of tuberculosis.

This review covers literature to date on tuberculosis as a disease and also as a problem in New Zealand. It covers the immunological aspects of infection with and protection against the disease. It also covers the concept of aerosol vaccination as a means of controlling disease in animals.

1.2. TUBERCULOSIS

1.2.1 Introduction to mycobacteria including tuberculosis

The genus *Mycobacteria* contain rod shaped acid fast organisms which are generally aerobic. They can basically be divided into slow and fast growing groups. Slow growers are divided into six distinct complexes, one of which is *M. tuberculosis*. There are four distinct species in *the M. tuberculosis* complex, *M. bovis*, *M. tuberculosis*, *M. africanum* and *M. microti* (Goodfellow and Wayne, 1982).

Robert Koch identified the tubercle bacillus in 1882 and the human and bovine types were differentiated by Smith in 1898. In the 1930s Wells emphasised the role of infectious droplet nuclei in the transmission of this airborne, inhaled pathogen (Jackson *et al.*, 1995). Human cases of tuberculosis numbered 3.4 million in 1997 and the number of new cases is increasing annually by about 100,000 (WHO, 1999).

1.2.1.1 Introduction to the general disease and host range of tuberculosis

In general terms an infection is the invasion and multiplication of microorganisms in body tissues, especially that causing local cellular injury due to competitive metabolism, toxins, intracellular replication, or antigen-antibody response. (Blood and Studdert, 1988). In addition, organisms causing respiratory disease such as tuberculosis must be able to reach the respiratory mucosal surface before establishing infection, usually by attachment to or penetration of the mucosal surface (Babiuk and Campos, 1993).

The progression of tuberculosis from infection to its various clinical manifestations can be considered as a series of stages, as the organism gradually invades further into the host. Initially the invading bacillus will multiply locally. This is followed by the formation of small caseous lesions which may grow and multiply into larger lesions that can shed bacilli into the blood and lymph systems. Caseous lesions may also liquefy and introduce bacilli and their products into the surrounding area, making arrest of the disease much more difficult (Dannenberg and Rook, 1994). Bovine tuberculosis has a very wide host range including humans, primates, carnivores, ungulates, marsupials, rodents and lagomorphs (Thorns and Morris, 1983).

1.2.2 Bovine tuberculosis

1.2.2.1 History in New Zealand

Bovine tuberculosis (tuberculosis) was probably introduced to New Zealand through imported infected cattle in the early nineteenth century at which time it was a serious public health problem, especially in children through ingestion of tuberculous milk (Pracy and Kean, 1969). In addition exports of beef and dairy products are majors sources of income for New Zealand, making it essential that international market requirements are met. The disease was controlled by implementation of test and slaughter schemes, initially for beef and dairy cattle in 1945 and then expanded to include deer in 1985 (Tweddle and Livingstone, 1994). These monitoring and control methods are now standard animal health and disease control procedures. Initially, the cattle scheme produced encouraging results but it was observed that despite apparently successful control, herds in some areas would revert back to infected status. In 1971 epidemiological evidence indicated that the Australian brushtail possum (*Trichosurus vulpecula*) was the cause of this reinfection (Hickling, 1991). The West Coast of the South Island was the first of these areas to be identified. Trapping of wild possums in this region revealed twelve percent of the population was infected with tuberculosis. The persistence of infection in dairy and cattle herds in the central and south eastern North Island was identified soon after for the same reason (ONeil and Pharo, 1995). Possum control programs were subsequently implemented, resulting in a substantial decline in tuberculosis levels by 1979. On the grounds of this success, funding for the control programs was reduced and this caused a resurgence of the disease as well as its expansion into previously uncontrolled areas (Livingstone, 1991). A review and subsequent increase in funding for disease control meant that by the end of 1991, 2.7% of cattle herds and 3.9% of deer herds were under movement control restrictions (Tweddle and Livingstone, 1994). By the year 2000 these figures had been further reduced to 0.8% of cattle herds and 1.8% of deer herds infected (Animal Health Board Annual Report, 2000). However, the area over which the disease may be found (vector risk area) has slowly increased over time to currently cover 33% of New Zealand, with a further 20% labelled a fringe testing zone. Annual expenditure on vector control, research and compensation to farmers increased slightly in 2000 to $52 million (Animal Health Board Annual Report, 2000).

1.2.2.2 Pathogenesis

Mycobacterium bovis is a facultative intracellular pathogen which multiplies within cells of the host's immune system, primarily macrophages (Aldwell *et al.*, 1996). There are five stages associated with infection with tuberculosis.

Sneezing and coughing are excellent methods of aerosol generation (Stark, 1998). The aerosols generated by tuberculous individuals may be contaminated with infectious bacilli which have an infectious distance of up to two metres (O'Hara, 1976). The first stage of infection is when contaminated aerosols are inhaled and reach the upper respiratory tract, in particular the tonsil and other lymphoid tissues, or the lower respiratory tract where they may adhere to the mucous membrane or lung surface.

Once the viable tubercle bacillus has adhered to the surface it is ingested and possibly destroyed by a macrophage. In the case of an *M. bovis* cell which reaches the lung alveolus, ingestion is by an alveolar macrophage. These are the body's primary mechanism for clearing inhaled foreign material. The level of bactericidal activation of the macrophage and the virulence of the ingested bacillus determine whether the bacillus is destroyed. When the macrophage does not have sufficient activity to destroy the bacillus, the bacillus will begin to replicate within the macrophage.

The second stage of infection is termed symbiosis and begins when the bacillus has replicated within the macrophage. This causes reduced viability of alveolar macrophages which may interfere with the ability of the immune system to recognise the invading bacilli (Aldwell *et al.*, 1996). It also causes the macrophage to spread and tightly adhere to the alveolar surface. Eventually the infected cell bursts and the now free bacilli will repeat the exercise in other activated and non activated macrophages, including those which migrate to the site from the blood stream. In time, macrophages from the circulation become completely responsible for the fate of the early lesion. Symbiosis begins here as the new macrophages ingest bacilli, but cannot inhibit or destroy them as they are not activated. The bacilli replicate logarithmically but do not harm macrophages because the host has not developed tuberculin-type hypersensitivity. With time the number of cells in this situation increase within the lesion (Dannenberg and Rook, 1994).

Stage three is the beginning of caseous necrosis. This starts when the rate of replication of bacilli in the core of the lesion declines sharply, usually 2–3 weeks after infection, and the host becomes tuberculin positive. At this stage, partially activated macrophages destroy the non activated ones in which bacilli have been replicating, causing caseous necrosis which is the formation of visible lesions. The host destroys its own tissue during this type of immune response. Bacilli cannot replicate in the caseous material due to environmental conditions and there is much bacillary material, or antigen present in the area. At this point the T cell mediated immune response first develops. In cattle lesions first become visible 5–6 weeks after bacilli are inhaled and excretion begins after approximately 14 weeks (Neill *et al.*, 1991).

Stage four involves the interplay of tissue damaging and macrophage activating immune responses in both susceptible and resistant hosts. In susceptible hosts, the bacilli released from the edge of the caseous centre are ingested by incompetent (non activated and poorly activated) macrophages. This means the host must continue its tissue damaging response to stop the intracellular bacillary multiplication. As a result the caseous centre grows in size and local lung tissue is destroyed. Bacilli draining into the lymph system are not destroyed and may initiate infection here. They may also drain from the lymph system into the blood stream resulting in the transport of infectious material throughout the body.

In resistant hosts bacilli are ingested by large numbers of activated macrophages which control bacillary replication. In this way bacilli are confined to the caseous centre and no additional self damage occurs. The disease is effectively arrested, frequently for the rest of the host's life although bacilli may survive for years in a dormant state in an apparently healed caseous focus.

The final stage of infection is liquefaction and cavity formation of existing lesions. This may occur even where there is a strong cell mediated response. The liquefied material is an excellent growth medium for bacilli and for the first time they may replicate extracellulary. In addition, it is toxic to macrophages. The liquefied material may be discharged into the airways, reaching other parts of the lung and acting as a source of infection to other hosts through aerosol transmission. It may also lead to pneumonia (Dannenberg and Rook, 1994).

1.2.2.3 Hosts in general

Many species may become infected with *M. bovis* (Thorns and Morris, 1983). Ungulates, particularly cattle and deer represent the greatest problem. The percentage of infected domestic cattle herds is highest in parts of Latin America (Argentina 37%, Chile 28.5% and Bolivia 18%) (de Kantor and Ritacco, 1994). There are also problem areas in Europe (Spain 10.7%, Ireland 8.8%) (Caffrey, 1994) with the badger (*Meles meles*) identified as an important host in Great Britain and Ireland (Cheeseman *et al.*, 1989). Tuberculosis is present in domestic cattle in both the United States and Canada. Elk, deer and bison from both wild and domestic populations have been recorded with tuberculosis in Canada (Munroe *et al.*, 1999). It has also been found in the captive deer industry in the United States (Essey and Koller, 1994). The spread of tuberculosis in a number of African wildlife game parks has been identified as a serious problem and threat to biodiversity. The key host is the African buffalo (S*yncerus caffer*) and disease has also been detected in several spill over hosts including the lion, cheetah, leopard and baboon (Buddle *et al.*, 2000).

Tuberculosis may occur in a range of rodents including the guinea pig, rat and mouse. Carnivores such as the cats, dogs and ferrets may also contract the disease (Thorns and Morris, 1983).

1.2.3 Wild hosts in New Zealand

Tuberculosis is present in several species of both wild and domestic animals in New Zealand. It is generally accepted that there are two reservoir host species, possums and deer. In these species the disease can be maintained by cycling within the population. There are also several spill over or dead end hosts (Hickling, 1995). The most important species is the possum.

1.2.3.1 Possums

The first recorded case of mycobacterial disease in a possum was in India in 1895 (Moore (1903) in Buddle and Young, (2000)). Tuberculosis was first identified in possums in New Zealand on the West Coast of the South Island in 1971 (ONeil and Pharo, 1995). In 2000 tuberculous possums were found over 24% of the South Island and 9% of the North Island, or one third of New Zealand’s total area (Animal Health Board Annual Report, 2000).

Prevalence of tuberculosis varies within possum populations but usually lies between 1–15% (Pfeiffer, 1994), (Jackson, 1995). However there are occasional outbreaks where prevalence is much higher, for example 60% in a low density possum population at Flagstaff Flat was recorded in 1992 (Coleman *et al.*, 1994). Two subsequent surveys indicated disease levels had decreased, with prevalence estimates of 17% and 9% (Coleman and Cooke unpublished 1995).

Tuberculous possums may be clustered in space and in time in areas colloquially known as hot spots (Coleman *et al.*, 1994), (Caley *et al.*, 1999). These hot spots may be set amongst large areas of possums with negligible levels of infection. Hot spots may be further divided into persistent sites, where tuberculous possums may be found over an extended time period, or sporadic sites, which are more likely the temporary effect of a single tuberculous possum which fails to transmit the disease to other possums at this site before dying (McKenzie and Morris, 1995).

Studies on the experimental infection of possums with tuberculosis were first carried out in New Zealand at the Wallaceville Animal Research Centre (O’Hara (1976) cited in Jackson and Morris, (1993)). Various studies have demonstrated direct and indirect transmission between possums while showing disease due to experimental infection to progress more rapidly than natural infection in the wild (Buddle and Young, 2000).

Buddle *et al.*, (1994) experimented with infection with very low doses (20 colony forming units) of *M. bovis* by intratracheal instillation. This resulted in loss of appetite in 3–5 weeks, followed by weight loss and clinical signs of disease. Severe tuberculous pneumonia was evident by eight weeks and some possums had died within 5–9 weeks. At post mortem, *M. bovis* was cultured from the lungs and lymph nodes of all possums studied with lesions, and also found in the liver, spleen and kidney.

Early hypotheses of major transmission pathways were centred around pasture contamination, however research has shown that this is not a significant factor (Jackson *et al.*, 1995). Transmission between possums in the wild is now thought to be primarily by aerosols (Lugton *et al.*, 1995). The minimum infective dose by aerosol is between one and three infective *M. bovis* bacilli (Lurie (1964) cited in Dannenberg and Rook, (1994)). Aerosols have been shown to be infectious over distances of up to two metres (O'Hara *et al.*, 1976).

There are three main pathways for transmission. The first path is direct horizontal aerosol transmission which occurs during agonistic or mating behaviour, particularly around den sites. The second route is indirect horizontal transmission which occurs when healthy possums become infected through using a den previously used by a tuberculous possum. The third pathway is pseudo-vertical transmission, where an infected dam transmits the disease to her joey through normal maternal behaviour (Jackson *et al.*, 1995). It is thought the majority of transmission between adult possums occurs during high levels of social interaction, for example mating behaviour or confrontations at or near den sites (Paterson, 1993), (McKenzie, 1999), (Pfeiffer, 1994).

In infected wild possums, disease progresses at a variable rate dependent on a range of stresses including environmental (notably adverse weather conditions), nutritional and the stress associated with lactation (Pfeiffer, 1994). Examination by (Lugton *et al.*, 1995) of 486 tuberculous possums showed that dissemination occurs during the early stages of disease and the infection of multiple sites is typical. Inguinal and axillary lymphocentres were most frequently affected and also showed evidence of infection in the very early in the disease process. This is probably due to their central role in lymphatic clearance of organisms. The lung was also commonly affected.

The disease process in possums was divided into four stages by (Lugton *et al.*, 1995). During the first stage there were no detectable gross lesions, however it is possible excretion via the respiratory tract was occurring. In the second stage some gross lesions were visible, particularly in the lung, and extensive dissemination of the disease was evident. The third stage was characterised by generalised disease with excretion primarily by the respiratory route, sinuses and mammary gland. However, there was no evidence of loss of body condition. In the short (1–8 weeks) final stage clinical effects of the disease develop rapidly and physical condition declines. Behavioural changes were evident, and included lethargy, weakness and poor coordination. Terminally ill possums may also be found wandering on pasture in daylight. Excretion via urine and faeces was noted, in addition to the routes already identified. In a study by (Pfeiffer, 1994) 50% of tuberculous possums survived for two months after detection of palpable lesions, and by five months 81% of possums with lesions were dead.

Geographic spread of the disease is thought to be primarily due to dispersal of tuberculous juvenile possums to uninfected areas. (Cowan *et al.*, 1996) monitored the dispersal of young possums and found 20% of radio collared individuals dispersed greater than two kilometres with some moving as far as 12 kilometres. The reproductive rate of the disease in possums is about 1.8 which means that each tuberculous possum is likely to infect two other possums in its life time (Caley and Ramsey, 1999).

The type of immunological reaction which the possum mounts in response to infection with tuberculosis appears to be ineffective, resulting in the rapid progression of disease. Possums can be grouped with ferrets and badgers in having an inability to wall off established lesions by formation of a granuloma. In the possum, this is a result of alveolar macrophages having an immunosuppressive effect on lymphocyte activity, regardless of whether the animal is infected with *M. bovis* or not. Cells which normally deal with bacteria are poorly organised to deal effectively with *M. bovis* (Buddle and Young, 2000). This is despite the fact that possum macrophages respond vigorously to infection (Jackson and Morris, 1993). Normally, the granuloma leads to containment and eventual control of the mycobacteria but in the possum, lack of granuloma formation leads to bacteria spreading to form satellite lesions, hence producing generalised and fatal disease (Buddle and Young, 2000). The disease in possums is typified by the presence of acid-fast organisms in large lesions with overt necrosis and microscopic granulomata with little or no necrosis. Infection is usually detected first in the lungs and associated lymph nodes, the bronchial lymph nodes and then the liver, spleen and kidneys as the disease invades the abdominal cavity (Buddle *et al.*, 1994), (Cooke *et al.*, 1995).

1.2.3.2 Deer

Deer (*Cervus* spp) were first introduced to New Zealand in 1851 (Challies, 1990) with the wild population now approximately 250,000 and a domestic population of 600,000 (Animal Health

Board Annual Report, 2000). Tuberculosis is present in both wild and domestic populations and enlarged lymph nodes about the head and neck are typical of clinical infection (Lugton *et al.*, 1995). Prevalence in domestic deer was measured at 1.8% in 1999/2000 (Animal Health Board Annual Report, 2000). Infection of deer with tuberculosis has been reported in other countries including North America, Great Britain, Ireland and Continental Europe (Lugton, 1997), (Munroe *et al.*, 1999), (Essey and Koller, 1994).

1.2.3.3 Ferrets

Ferrets (*Mustela putorius*) were first introduced to New Zealand in 1879 to help control the rabbit population and also in captivity to start a fur industry. The wild population is now widespread throughout New Zealand though densities are highest where rabbit numbers are high. Ferrets are regarded as pests, mainly as a result of damage to native animals, particularly flightless birds. The diet of the ferret is mainly small mammals, supplemented with carrion. It is probable that wild ferrets became infected with tuberculosis through consumption of diseased carrion, for example possums and domestic animals discarded in offal pits (de Lisle et al 1993). Tuberculosis was first reported in ferrets in the 1970s (Stockdale, 1975) and their importance and relationship with possums in regard to the disease has been the subject of much debate (Ragg *et al.*, 1995). However, it is now becoming accepted that ferrets are not true maintenance hosts for the disease (Caley *et al.*, 1996). Prevalence of disease ranges from 15% (Ragg and Walker, 1996) up to 90% (Lugton *et al.*, 1995) and signs of infection are typically lesions in the mesenteric lymph nodes and pulmonary region (Dunkin (1929) cited in Lugton, (1997)).

1.2.3.4 Feral pigs

Feral pigs (*Sus scrofa*) are widespread throughout New Zealand. Wild pig hunting is a popular recreation and populations are maintained in some areas by the release of young pigs. The omnivorous, opportunistic feeding habits of feral pigs are well suited to consuming potentially tuberculous carcasses found in the wild. Pigs infected with tuberculosis can be found in areas where the disease is considered endemic in other species. Prevalence of disease was measured at 31% in Central Otago (Wakelin and Churchman, 1991). Disease is characterised by lesions in the lymph nodes, which regress as the pig ages (Ray *et al.*, 1972).

1.2.3.5 Hedgehogs

In New Zealand, hedgehogs (*Erinaceus europaeus*) are abundant in developed lowland districts but less common in forest environments and mountainous areas (Brockie, 1990). Recorded cases of disease are uncommon with lesions occurring primarily in the lungs and lymph nodes (Lugton, 1997).

1.2.4 The epidemiology of bovine tuberculosis in New Zealand

The epidemiology of tuberculosis in New Zealand is discussed in three parts. Aspects of the disease are covered first, followed by susceptible hosts and finally, important environmental factors.

Tuberculosis is a mycobacterial disease spread mainly by aerosol and caused by *M. bovis*. It is predominantly a disease of the respiratory tract, though spreads throughout the body in advanced stages. *M. bovis* is an obligate pathogen but can survive for variable periods in the environment. The duration of survival is influenced mainly by temperature, moisture, pH and exposure to sunlight (Morris *et al.*, 1994). The three most important environments for survival in New Zealand are possum den sites, pasture and the carcasses of animals which have died of tuberculosis. In possum dens, survival of the organism ranged between seven and 28 days. On the forest floor survival was less than four days in summer and between 14 and 28 days in winter. *M. bovis* survived less than four days on pasture regardless of season (Jackson *et al.*, 1995). Survival of the disease in unscavenged carcasses of dead hosts was at least four weeks, although most accessible carcasses were scavenged completely within two to three days (Pfeiffer and Morris, 1991).

There are a number of different strains of *M. bovis* that can be identified by restriction endonuclease (REA) typing, though there is no evidence of any difference in virulence between these strains (Morris *et al.*, 1994).

The key hosts in New Zealand are domestic cattle and deer, wild deer, possums, ferrets and pigs. Transmission between hosts, other than the scavenger species, is primarily by aerosol. The majority of spread is between possums and this is hypothesised to occur mainly around den sites (Pfeiffer, 1994). Long distance spread of the disease is largely due to dispersal of young possums seeking new home ranges and the commercial trading and transport of infected livestock (Pfeiffer, 1994). Transmission from possums to cattle and deer occurs when the possum is terminally ill. The disease causes the possum to become weak and lethargic and to move around during daylight. This erratic behaviour stimulates active investigation by deer and cattle. The normal response of a possum to such threats is a loud hissing screech which is ideal for generating infectious aerosols, which may be inhaled by investigating animals (Paterson and Morris, 1995). There is a smaller amount of spread within cattle and deer populations. Ferrets become infected primarily through the alimentary tract as a result of consumption of tuberculous carcasses (Caley *et al.*, 1996). Feral pigs can also become infected through consuming tuberculous carrion though the disease process is often arrested by the pigs immune system (Ray *et al.*, 1972).

Hosts may be divided in to two categories. The first category, maintenance hosts, represents species in which the disease is self-sustaining. Possums and possibly deer are examples of wild maintenance hosts (Hickling, 1995). The second category, spill over hosts, represent species which may become infected with tuberculosis, but cannot sustain infection long-term within the species. In the absence of an ongoing external source of infection (for example the possum), disease will die out in these species. Examples of spill over hosts in New Zealand include ferrets, wild pigs and wild deer (Morris *et al.*, 1994).

There are several environmental factors which are important in the persistence of tuberculosis in New Zealand. Farmland which contains or borders on suitable possum habitat is most likely to be associated with a tuberculosis problem. The area where bush or scrub and pasture meet is a prime example of high risk habitat (Caley and Coleman, 1998). In addition infection is frequently clustered both in space and time in possum populations (Coleman, 1988). Peaks in the level of tuberculosis recorded in possums coincide with peaks in adjacent cattle, suggesting disease in one species triggers that in another (Coleman *et al.*, 1999). This suggestion is supported by DNA fingerprinting studies which show that often the same strain of *M. bovis* is infecting both species (Collins *et al.*, 1988).

Tuberculosis can only be eradicated from New Zealand by controlling infection in both wildlife and domestic stock (Buddle *et al.*, 2000). Although levels of disease in livestock can be maintained at very low levels, the inability to control disease in wildlife will result in continued patchy outbreaks of disease (Morris *et al.*, 1994). Currently New Zealand’s test and slaughter strategy has reduced infection to 0.8% of cattle herds and 1.8% of deer herds. Approximately $30 million is spent annually on vector control (primarily possums) in the form of poison baits and trapping. This is part of the $50 million spent in total on the tuberculosis problem annually (Animal Health Board Annual Report, 2000).

1.2.5 Tuberculosis in the badger

In Great Britain and Ireland, the badger (*Meles meles*) is associated with the persistent infection of livestock with tuberculosis, in much the same way as the possum in New Zealand. In Great Britain the first tuberculous badger was identified in 1971, after investigation of unexplained outbreaks of the disease in cattle. As a result, badger culling operations were implemented to control the disease, in addition to existing control methods. Culling operations reduced rates of infection in cattle in localised areas, but failed to eradicate the disease. It is now known that tuberculosis is endemic in badgers in some areas, and that they function as a wildlife reservoir for disease (Cheeseman *et al.*, 1989), (Little *et al.*, 1982). Badgers are omnivorous, feeding mainly on earthworms, and live in groups of up to 15 animals in underground setts. The dark, confined conditions within a sett provide excellent conditions for the transmission of tuberculosis (Roper, 1992), (Clark, 1988). Prevalence of disease over six years in Gloucestershire varied from 0% to 8% (Cheeseman *et al.*, 1989). Characteristics of infection in badgers include lesions in the respiratory and urinary systems, and associated lymph nodes. Suppurating abscesses may result from infection via bite wounds, sustained during agonistic interactions (Smith *et al.*, 1995). Healthy badgers avoid contact with cattle and rapidly move away if approached (Benham and Broom, 1989). However, the behaviour of badgers in the terminal stages of disease is similar to that of terminally ill possums, for example physical debilitation, lethargy, being seen abroad during daylight and not showing normal avoidance behaviour (Jackson, 1995). The sputum, urine and faeces of tuberculous badgers have been shown to contaminate pasture (Smith *et al.*, 1995) but the most important route of transmission to livestock is still unclear. Increasing public disapproval of badger culling indicates that alternative methods for the control of tuberculosis in badgers and cattle are required (Clifton-Hadley, 1996). One possibility is the vaccination of badgers with BCG. Vaccination of badgers enhanced cell mediated immunity, delayed excretion of the organism and prolonged survival of infected badgers (Stuart *et al.*, 1988).

1.3. THE POSSUM

1.3.1 General introduction

The Australian brushtail possum (*Trichosurus vulpecula*) is a member of the Order Marsupalia; Family Phalangeridae. Adults weigh between two and four kilograms and are approximately the size of a domestic cat. The possum is nocturnal, principally arboreal and usually solitary. Possum populations are widely distributed within Australia and Tasmania where it typically inhabits open eucalypt woodlands, competing with a number of other arboreal marsupials (Clout and Ericksen, 2000).

The possum was first successfully introduced into New Zealand in Riverton on the west coast of the South Island in 1858 (Pracy, 1974). Numerous releases of possums were made during the 1890s primarily to start a fur trade. Government protection and active distribution of the possum was used to facilitate colonisation up until 1947. By this date public concern over environmental damage was sufficient to put a ban on all further releases and by 1951 a bounty scheme was instigated to encourage the destruction of the possum (Jackson and Morris, 1993). Today, possums number approximately 70 million (Cowan and Bayliss, 1998) and occupy about 95% of New Zealand. They are found throughout a wide range of habitats (Pracy, 1974) and are considered a serious environmental pest (Morris *et al.*, 1994).

The possum is regarded as a serious pest for various reasons. First and foremost it acts as a reservoir and spreading agent for tuberculosis. This is discussed in depth elsewhere (section 1.2.4.1). Secondly, the sustained browsing of some native tree species by possums leads to tree death and in some cases complete collapse of the native canopy. It may also cause a change in floristic composition of the plant population, which can occur in as little as 15–20 years. Rata and Kamahi forest areas in the South Island are prime examples of possum damage (Payton, 2000). Thirdly they are predators of native animals. Possums have been shown to eat the eggs, chicks and even adult birds of a range of native species including the kiwi. They have also been found to prey on native snails and a range of insects (Sadlier, 2000). The final reason why the possum is regarded as a pest is their economic impact on New Zealand’s primary production. Feeding damage has been recorded in at least 46 varieties of fruit and vegetable, although damage may be seasonal and patchy. In addition, the growth of young stands of *Pinus radiata* is reduced by possum browsing (Butcher, 2000) and willows and poplars planted for erosion control and catchment protection are also frequently damaged (Thomas *et al.*, 1984).

The possibility of possum farming has been considered in the past with the primary products being fur, pelts and meat. This idea was not successful due to changes in possum behaviour resulting from captivity, in particular the failure to breed (Leech, 1979).

1.3.2 Possum biology

The possum is highly adaptable. So variable is the possums behaviour that observations and conclusions from one locality may be irrelevant when considering populations elsewhere (Cook, 1981). This adaptability is evident in a wide range of biological factors such as breeding, feeding and denning habits and population density which is usually between 1–10 possums/hectare (ha) but has been recorded as high as 25 possums/ha (Coleman *et al.*, 1980). The main breeding season is from March to June but in some areas there is also a smaller spring breeding season (Fletcher and Selwood, 2000). Possums eat a wide range of food which varies with habitat and season. Major constituents of the diet include foliage, pasture and seasonal foods such as flowers and fruit (Nugent *et al.*, 2000).

1.3.2.1 Possum home ranges

Studies in a variety of habitats have determined the size of possum home ranges and degree of overlap of individual ranges. A summary of the findings is presented by (Cowan and Clout, 2000).

In Australia home ranges were found to be on average 3 ha for males and 1.5 ha for females. Female ranges would frequently overlap, while male ranges were exclusive (Dunnet, 1963). In New Zealand extensive overlap of possum home ranges is common both between and within sexes (Crawley, 1973).

In a dense native bush environment such as the Orongorongo Valley (lower North Island, New Zealand), possum density was found to be 10.6 possums/ha and their home range to be 0.8 ha for males and 0.5 ha for females. In a pine plantation the home range was estimated at 0.7 ha for both males and females (Warburton, 1977). In mixed bush, scrub and pasture habitat such as in the Wairarapa, home ranges were estimated at 1.4 ha for males and 0.9 ha for females (Paterson *et al.*, 1995). Within the home range can be found one or possibly several smaller foci which constitute a denning range (Ward, 1978). Where land is very steep and especially at high altitudes possum home ranges extend further vertically than horizontally because a greater range of plant types and hence food types are covered by changing elevation (Green and Coleman, 1986).

1.3.2.2 Preferred habitat and the bush/pasture margin

Provided there are sheltered den sites and adequate food sources, possums live almost anywhere in New Zealand. They are found in native and exotic forests, grasslands, sand dune and swamp country, even urban and city areas. Faecal signs have been recorded from sea level up to 2,400 m (Pracy, 1974). Possums may be found in unusually high densities along the area where bush or scrub and pasture meet (bush/pasture margin). This is because bush and scrub habitat provide many suitable den sites while pasture provides a favoured source of food (Green and Coleman, 1981). Possums have been recorded moving up to 1,000 m through bush to pasture. These movements were found to coincide with peaks in pasture growth, indicating it is an important source of food. Once possums had moved from the bush to pasture, they seldom moved further than 300 m away from the edge of the forest (Green and Coleman, 1986). The significance of pasture as a constituent of the diet of the possum is disputed by Harvie (1973) and Jolly (1976) who recorded possums feeding on pasture seven times, fruit trees 250 times and willow trees 80 times.

Studies have found an unusually high prevalence of tuberculosis in possum populations on the bush/pasture margin (Coleman, 1988). This habitat was previously thought to be an important factor in the transmission of disease from possums to cattle and between possums. It was believed that diseased possums would contaminate pasture, which was subsequently grazed by cattle and healthy possums. Transmission was also thought to result from frequent interactions between diseased and healthy possums on pasture (Ekdahl *et al.*, 1970). However, more recent studies have shown that pasture contamination is an unlikely route for disease transmission. The proportion of cattle infected via the alimentary route was suggested as very small by Morrison *et al.* (2000) with most tuberculous cattle having a lesion distribution indicative of infection via the respiratory tract. In addition, the survival of *M. bovis* in a pasture habitat is unlikely to be more than four days (Jackson *et al.*, 1995). Observations of possums on pasture by Paterson *et al.* (1995) and Brockie (1987) showed very few interactions close enough to facilitate aerosol transmission.

1.3.3.3 Ranging characteristics

The young adults of many wild mammalian species disperse away from the territory of their parents in order to establish their own. This natural tendency facilitates geographic spread of possums into new areas. Young possums disperse between approximately ten and 24 months of age (Jackson and Morris, 1993). A greater proportion of males disperse, though on average females disperse further and movements of greater than 20 km have occasionally been measured (Brockie *et al.*, 1987). Where the dispersing possum is infected with tuberculosis, these movements are likely to result in geographic spread of the disease. Once a home range is established, possums usually remain there for life (Crawley, 1973).

Possums may make long distance forays, measured at up to 1,600 metres, to seasonal food sources such as apples, walnuts, pine catkins or the spring growth on a range of species including poplar and willow trees (Jolly, 1976).

1.3.3 Possum behaviour

Patterns of intraspecific social behaviour influence the fundamental actions of a species, such as mating, feeding and competition for shelter, to ensure the persistence of that species (Grier, 1984). The aspects of possum behaviour were covered in broad detail by Montague (2000). There are three particular aspects of possum behaviour which will be addressed in this review. These are neophobia, social hierarchy and interactions between possums around traps or bait stations.

1.3.3.1 Neophobia

Neophobia is the fear of novel objects, causing avoidance of new, potentially dangerous stimuli within a familiar environment, thereby ensuring the safety of the animal. It is an important component of behavioural ecology. The degree to which this aversion is present varies due to a range of factors including number of previous presentations of the novel stimulus, sex and social rank of the subject, and even individual identity (Sunnucks, 1998).

Minimal neophobia is evident in wild possums. In contrast to avoidance, (Short, pers. com., 1998) claims investigatory behaviour toward novel objects by wild possums to be very active and aggressive, in comparison to captive possums. Formal studies have also shown possums to be naturally curious, and to actively investigate novel objects (Carey *et al.*, 1997). In comparison with other marsupials, possums show very little wariness or avoidance behaviour (Russell and Pearce, 1971).

Neophobia is present in some wild possums in the form of bait and poison avoidance. The major cause of this is previous sub-lethal doses of poison. Avoidance of poison baits was shown by 68% of mature possums trapped from a previously poisoned area. Approximately 20–25% of possums also rejected poison baits during their first exposure, and some were found to avoid novel non-toxic baits. It is unclear whether this behaviour is learned or innate. Some of these animals continued to demonstrate avoidance after two and a half years. In comparison, bait avoidance was not demonstrated by young possums trapped in a poisoned area (Matthews and O'Connor, 1996). (Morgan, 1990) showed that although up to 34% of possums rejected toxic pellet baits, non-toxic baits were rejected by only 5–7% of possums.

1.3.3.2 Attractants

Possums are solitary and nocturnal. They have a large olfactory bulb, well developed vomeronasal region and many scent glands indicating scent is an important component of possum ecology (Todd, 1995). Of particular importance are those scents used for individual recognition, allowing subordinate animals to avoid areas occupied by dominants (Day *et al.*, 1998).

Effective lures have been found for a number of mammals. They usually contain compounds recognised by the target animal, for example favoured seeds of deer mice and rice strains preferred by rats (Morgan *et al.*, 1995). Olfactory lures are the traditional equipment for attracting possums and virtually every imaginable material has been tried at some time with highly variable results (Cowan, 1987), (Pracy, 1974), (Morgan *et al.*, 1995). The failure of the trial by Todd (1995) to identify a favourite amongst twenty attractants can be regarded as a typical result. To complicate matters further there are many hunters who are convinced of their own particular formula, while other experienced hunters note the cyclic trends in popularity of the more common types of lure. Although anecdotal, the views of trappers have value. Their beliefs have a less scientific basis than experimental trials but they depend on the effectiveness of their compounds for their livelihood. The results of scientific trials are only slightly more informative. White light and an electronic beep have been trailed as alternatives to olfactory lures. Results showed that a proportion of possums would not approach either of the stimuli. For those possums which did investigate, there was no significant difference in approach or investigation between treatment and control boxes (Carey *et al.*, 1997). White material, for example flour, is reported to function as a visual lure possibly by imitating the white precipitate left after possum urine has dried. However, live trapping studies have found that use of a lure increases the total number of possum catches, but neither constant use of a single lure, nor daily changes of lure increased the proportion of the population captured each month (Cowan, 1987).

1.3.3.3 Social behaviour in the possum

A social hierarchy is present in a wide range of animals including the possum. This hierarchy or ‘pecking order’ determines how resources such as food, shelter and mates are distributed amongst a population. When resources are abundant, they may be used by all levels of the social order, however when resources are scarce, those at the bottom of the order go without. The ranking of individuals in a social order is determined primarily by agonistic interactions (Grier, 1984).

The social order of possum populations has been the subject of several studies. In both New Zealand and Australia social order is based on a system of mutual avoidance by co-dominants of both sexes. Dominance is probably established by initial encounters between individuals and is thereafter maintained mainly by memory (Winter, 1976). Social postures and agonistic interactions while defending den sites also maintain this hierarchy (Biggins and Overstreet, 1978). Winter (1976) studied wild possums and concluded that there was a linear hierarchy for each sex and that the order of individuals was influenced primarily by age and body weight. The idea that a hierarchy is determined by age and body weight is supported by both (Jolly, 1976) and (Oldham, 1986). Oldham also reports that interactions between males were more frequent than between females and that in a mixed sex colony, females were usually dominant over males.

In contrast, an earlier study reported social order in the possum to be of little if any value. It also claimed that the lack of conventionalised dominance and submission prevented group formation, since the response to aggression in possums is fight or escape (Kean, 1967).

The pattern of older, heavier individuals occupying the dominant positions and females being dominant over males has been found in the red kangaroo (*Megaleia rufa*) (Russell, 1970). In the grey kangaroo (*Macropus giganteus*) males were found to be dominant in captivity and in the wild, and a social order was present in both males and females (Grant, 1973).

Wild possums at bait stations show mutual avoidance of cospecifics. Hickling and Thomas (1990) report that feeding possums were alert to the presence of other possums nearby, but that bait stations were never used by two adults at the same time. If a possum approached a bait station that was in use, it would either stop 5–10 metres away and wait for it to be vacated, or the new arrival would continue to approach until the possum at the station stopped feeding and moved away. There was no direct contact between males, though clumps of fur were occasionally found around heavily used stations.

1.3.4 Possum control

The first efforts to control the possum population New Zealand began in 1951 with a bounty scheme (Jackson and Morris, 1993). Control operations have been carried out regularly since then for the protection of livestock against tuberculosis and the protection of native forests. The primary method of control is the aerial distribution of diced carrot or grain based pellets to which sodium monofluoroacetate (compound 1080) has been applied (Morgan, 1990). Maintenance control operations, based on manual poisoning and trapping techniques, must also be performed every one to two years, (Department of Conservation, 1999). In the year to June 2000 Animal Health Board expenditure on possum control was $27 million. An additional $2.5 million is invested in research for more effective methods of possum control (Animal Health Board Annual Report, 2000). While the existing control methods are effective at reducing possum numbers in the short term, areas can be recolonised as a result of the vacuum effect (see section 3.5.1). Overall they have had little success at reducing the area of New Zealand in which tuberculosis is endemic. There are increasing public concerns over the continued use of large quantities of poisons and the on going financial cost of control schemes (Buddle *et al.*, 2000). In addition, possum traps and poisons are a hazard to other wildlife including New Zealand’s native flightless birds (Warburton *et al.*, 1997).

Three aspects of possum control are relevant to this review. These are the use of bait stations and the concepts of the vacuum effect and buffer zones.

1.3.4.1 The vacuum effect

The vacuum effect is a term used to describe the permanent movement of possums from their original home ranges in uncontrolled areas to adjacent regions which have been subject to control operations. Following control, possums in the surrounding area are attracted into the depopulated area by available den sites and increasing food resources as plant life recovers from possum browsing. This movement is distinct from juvenile possums immigrating in search of their own home range. The vacuum effect has been described in the past as a key part of possum recolonisation, particularly in the two years following poison operations and in areas where maintenance control is not carried out (Cowan and Clout, 2000). However, a number of studies indicate that the vacuum effect may not be as important as commonly believed. Green and Coleman (1984) showed very few possums moved into a 100 ha area up to three years after intensive control operations. (Cowan, 1993) studied possums on Kapiti Island and also found no evidence of movement following intensive trapping operations. (Efford *et al.*, 2000) found some changes in home range, though adjustments to individual ranges were usually only about 50 metres into the controlled area.

1.3.4.2 Buffer zones and initial control

A buffer zone is a perimeter of particular width around a previously controlled area, or area of importance, farms for example, which is maintained at a very low possum density. The effect of this is to stop the possum population outside the buffer zone recolonising the controlled area within, essentially creating a possum free ‘island´. Where possum and cattle populations are infected with tuberculosis, farms placed within these ‘islands’ have a much greater chance of eradicating the disease from livestock.

Buffer zones are initially created by control operations designed to reduce the possum population by 70–90% as determined by the residual trap catch of 5% or less, which is the standard Animal Health Board target for success of a control operation. This makes the assessment of success dependent on initial possum density (Livingstone, pers. com., 1999). Following initial control, maintenance control is used to keep the buffer zone below 40% of the pre-control population as recommended by (Nugent *et al.*, 1997).

In a study on the effectiveness of a buffer zone, the percentage of reactors from farms adjacent to forest buffer widths of 1, 3 and 7 km were compared. The effect of these controlled buffer zones was to lower the incidence of tuberculosis from 1.22%–2.06% down to 0.35%. This reduction was not significantly different across the three buffer widths. After two years, the possum population in the 1 km buffer zone was recovering rapidly, particularly around the forest edge of the buffer, while in the 3 and 7 km areas it remained low. The study concluded that wider buffer zones were more effective at maintaining possum populations at low levels for longer periods, though the cost relative to width of buffer must also be taken into account (Fraser *et al.*, 1998). Replication of this trial would be beneficial to confirm Fraser’s results, as it is the only substantial research into the effects of buffer zones on possum populations and reactor rates in New Zealand.

1.3.4.3 Bait stations

Bait stations are small plastic weatherproof containers which are used for dispensing pre-feed and poisons for possum control. They are becoming increasingly important in the ground based control of possums. They can also be used to provide useful information about the local possum population. This information can be divided into three types. Firstly, the size of the possum population in the immediate area can be determined by the volume of bait taken as each possum consumes on average 100 g of bait (Hickling and Thomas, 1990). This information can be used to calculate the volume of poison required to efficiently control a range of possum densities.

Secondly, observation of bait stations used by wild possums has improved the understanding of behaviour around fixed feeding stations. It has also shown the effect of bait station density on proportion of the possum population using the devices. Investigation in four habitats showed the number of possums attending increased rapidly over the first two nights then levelled off (Hickling and Thomas, 1990). Between 90–100% of possums within 50 m would visit a bait station and 60–70% of possums within 500 m (Morgan *et al.*, 1995). In another trial bait stations spaced at 100 m intervals were used by more than 90% of the possum population. The proportions of possums using bait stations at 100 m and 150 m were not significantly different from this, though there was a significant reduction in use at 200 m spacing (Thomas and Fitzgerald, 1995). Thomas *et al.*, (1996) found that bait stations spaced at 150 m along forest-pasture margins were used by 79% of possums captured within 300 m of bait stations. In the forest this figure was over 80%. On the strength of these results Hickling and Thomas (1990) recommend that feeder stations should be spaced no more than 100 apart.

Thirdly, when used as pre-feeders they can be used to train resident possums to frequent a particular area. It is likely that repeated food rewards from a bait station would reduce fear of investigating these novel objects. Pre-feeding with bait stations has been shown to increase cyanide kills (Morgan *et al.*, 1995). It has also been shown to increase the amount of 1080 taken from bait stations on the first night and overall by 70% (Thomas, 1998). Bait acceptance from stations has been shown to vary little (85–100%) between seasons, thought to be slightly lower at the bush pasture margin (84%) than deeper in the forest (95%). The suggested reason for this is that pasture species are more nutritious than forest species and the result of this is reduced interest in additional food sources (Morgan *et al.*, 1999).

In contrast to this data, Keber (1987) cited in (Hickling and Thomas, 1990) found that bait stations had little effect on the movement of possums, except for those animals which would have encountered the station either in the course of normal movements about their ranges, or as a result of occasional long distance movements. Bait station use was unaffected by the use of cinnamon oil as an attractant (Morgan *et al*., 1995).

1.4. IMMUNOLOGY

1.4.1 Introduction to immunology

The mammalian immune system protects the body against foreign material (Blood and Studdert, 1988). This includes pathogenic microorganisms such as bacteria, mycoplasma, virus and fungi (Villee and others, 1984). There are two key divisions to the immune system, non specific and specific immunity. Specific immunity is further divided into cell mediated and humoral immunity (Kuby, 1997). A particular immune response may involve a single immune process, or a combination of different immune processes. This review focuses on those aspects of immunology relevant to infection with airborne pathogens and begins with a discussion of respiratory tract and mucosal immunity, followed by cell mediated immunity and localised immune responses. The immune response particular to tuberculosis concludes this section.

1.4.2 Non specific immune mechanisms

Non specific immunity is the basic resistance to disease which an individual is born with. It utilises four types of defensive barrier, anatomic (skin and mucous membranes), physiological, (low pH of the stomach, temperature and chemical mediators) phagocytic (macrophages) and inflammatory. It is the first immune defence against foreign material and destroys most microorganisms within a few days (Klein and Horesji, 1997).

1.4.2.1 Upper respiratory tract

The upper respiratory system is the point where an inhaled pathogen first enters the body. It has several functions including acting as a conduit for air during respiration and a non specific immune mechanism by using mucous membranes to filter and condition the air. It is also responsible for olfaction and phonation (Beech, 1991).

Inspired air is contaminated with a wide range and varying amount of airborne particles including microorganisms, dust, smoke and soot. The average human inhales about 10,000 organisms daily (Klein and Horesji, 1997). The respiratory tract is composed of many sharp twists and turns (bifurcations) cause turbulent air flow during inhalation and exhalation. This turbulence causes the larger particles to be thrown against the mucous membrane or mucus covered hairs, to which they adhere. The nasal surface receives the largest amount of foreign material, although the physiological effect of material deposited here is minor compared with the small amount penetrating further into the airways (Knight, 1973). The mouth and throat are protected by a constant flow of saliva which washes deposited particles to the stomach (Klein and Horesji, 1997).

1.4.2.2 Mucociliary system

Mucous secreting membranes are present in the upper respiratory tract, conjunctiva, vagina and intestinal tract (Mestecky and McGhee, 1989). Mucus is derived from goblet cells and defined as the free slime of the mucous membrane. It consists of fluid, salts, leukocytes (lymphocytes), plasma cells, glycoproteins, desquamated cells, various salts, and polysaccharides. It is the long nature of this final constituent’s molecular structure which makes mucus viscous (Villee and others, 1984), (Blood and Studdert, 1988). Mucous membranes are covered by a continuous blanket of mucus. This blanket has a thin ‘sol’ top layer and thicker ‘gel’ layer underneath. The blanket acts as a dynamic clearing mechanism, with the mucus propelled by ciliated cells in metachronal waves. Particles which land on the cilia stimulate production of a ‘raft’ of mucus (Hatch and Gross, 1964) which then carries the particle at a rate of approximately 18 mm per minute (Antweiler, (1958) cited in Hatch and Gross, (1964)) toward either the pharynx or larynx (Tyrell, 1972;Beech, 1991). From here the particle is swallowed and destroyed in the acidic environment of the stomach (Hensel, 1996). The physical properties of mucus and the efficiency of tracheal clearance depend on maintenance of the balanced interaction among several cell types (Basbaum, 1984). It is possible for the inhaled particles to cross this mucosal barrier and this is by way of M cells, which transport antigens from the surface into the body to lymphoid tissue for destruction (Klein and Horesji, 1997). Very high levels of air contaminants reduce the clearing capacity of the respiratory tract and this can increase the risk of lung infection (Cox and Wathes, 1995).

Overall, mucous membranes protect a very large surface area of the body against foreign material. However they are also the most frequent routes of entry for bacterial, viral and parasitic infection (Mestecky and McGhee, 1989). The mucociliary system is present throughout the upper airways but does not protect the lower respiratory tract.

1.4.2.3 Lower respiratory tract

Protection against airborne pathogens in the lower respiratory tract is the responsibility of alveolar macrophages (West, 1987). Macrophages are large phagocytic cells derived from white blood cells. They are found in many of the body’s tissues including the alveoli of the lung (Villee and others, 1984). Alveolar macrophages act as scavengers, roaming around the surface of the alveoli and engulfing particles which have landed on the lung surface from inspired air. Most of these particles are dead but some are pathogenic. The majority of these particles are digested *in situ* (Klein and Horesji, 1997) although in some cases macrophages, having ingested the particle, leave the lung via either the lymphatic system or blood stream. They may also migrate to the mucociliary system where they are transported to the stomach and digested or alternatively, expelled from the body by coughing (West, 1987).

Macrophages transport antigenic material and present it to lymphocytes. This is an important link from the non specific immune system to the specific immune response (Beech, 1991).

1.4.3 Specific immune mechanisms

In some situations a microorganism is not contained by the non specific barriers. When this occurs the specific immune system works together with the non specific process to control the invading microorganism. The basic building block of a specific immune response is the lymphocyte which is derived from white blood cells. There are two main groups of lymphocytes, the B group and the T group (Kuby, 1997).

Lymphocytes actively identify and adapt to control an enormous range of foreign invaders (antigens). The reaction to each antigen is highly specific. Lymphocytes also maintain a long lasting memory of each antigen to which they are exposed. This memory allows much faster, more potent and longer lasting control of any antigen previously encountered. Lymphocytes are able to differentiate between self and non self (Kuby, 1997).

The specific immune mechanism is divided into two categories, the cell mediated response and the humoral response. These areas are distinct, although there is much interaction between the two. This is because a localised immune response can expand into a systemic response and usually involves communication between different parts of the body (Klein and Horesji, 1997)

1.4.3.1 Cell mediated *immune response*

A cell mediated immune response uses T lymphocytes which develop in the thymus. The T group comprises two subpopulations – helper T cells and cytotoxic T cells. Helper T cells are the major antigen recognising cells in almost all specific immune reactions. Their function is to activate the body’s phagocytic cells when the appropriate antigenic material is encountered. They do this by secreting cytokines which lead to the excitement of macrophages, immunoglobulins and cytotoxic T cells (Kuby, 1997). Activation of macrophages is the major part of a cell mediated immune response and consists initially of their proliferation. The increasing number of macrophages also undergo changes in their cellular properties including size, speed, energy consumption, enzyme production and phagocytosis. This group of activated phagocytic cells are much more capable of antigen destruction. They remain local to the site of the infection while other macrophages may also migrate to the site of infection from the blood stream as a result of inflammation (Dannenberg, 1968). The magnitude of a cell mediated immune response is dependent on a number of factors, for example regional differences within the organ system, age, species and the virulence mechanisms of particular pathogens (Wilkie, 1982).

Cell mediated immunity is used to control infection with tuberculosis. The size of the cell mediated immune response of animals exposed to *M. bovis* can be measured by the lymphocyte stimulation assay (Buddle *et al.*, 1994). The disease process is covered in detail in the section 2.2.2.

1.4.3.2 Humoral immune response

A humoral immune response acts on antigen found in the blood stream, in contrast to a cell mediated response which acts at a specific location. A humoral immune response involves the B group of lymphocytes (antibodies). B lymphocytes develop in the bone marrow and further differentiate into immunoglobulins (Ig) mainly on the intestinal mucosa and also in the bone marrow, spleen and lymph nodes (Brandtzaeg, 1989). There are five main classes of immunoglobulin of which IgG is the major type and found in the blood (Nugent and Lugton, 1995). IgA is found in mucus of the respiratory tract**,** intestinal tract, tears, sweat, saliva and milk. The function of these immunoglobulins is the highly specific recognition of antigenic material. On recognition of the appropriate antigen, the antibody binds to it, triggering events which lead to destruction of the antigen (Villee and others, 1984), (Roitt, 1998).

1.4.3.3 Immune response to mycobacteria and in particular tuberculosis

The respiratory tract is the most important route of initial infection for tuberculosis, however as disease progresses lesions may also be found throughout most of the internal organs (Buddle *et al.*, 1994;Cooke *et al.*, 1995;McCool, 1979). Inhaled aerosols containing infective bacilli vary in size. Large aerosols (8 ?m or more) become attached to the mucous membrane of the upper respiratory tract. Movement of mucus will carry the particle to either the pharynx or larynx where it will be swallowed and destroyed in the acidic environment of the stomach (Hensel, 1996).

Smaller aerosols generally do not become caught on the mucous membrane and penetrate further into the respiratory tract to the lung. The size best suited for maximum penetration is 4–5 ?m

(Walker and Stephen, 1977) and particles of this size contain a maximum of three bacilli (Lurie (1964) cited in Dannenberg and Rook, (1994)). A proportion of these particles will remain suspended in the air and be exhaled, but others may adhere to the lung surface. Those adhering to the lung surface will then be engulfed by alveolar macrophages. While the action of engulfing usually results in destruction of the invading organisms, in some cases the enzymes within the macrophage fail to do this. When the macrophage fails to destroy an infective *M. bovis,* the bacillus may then proliferate within the cell. This proliferation causes the cell to swell and eventually burst at which stage many bacilli are released to infect more macrophages. The number of macrophages infected with bacilli and tuberculin like products (antigen) increase logarithmically (see section 2.3.2) for 2–3 weeks to the point where they become an irritant to the body. Helper T cells react to the irritant by activating macrophages, immunoglobulins and cytotoxic T cells (Kuby, 1997). The activated macrophages attempt to control the disease by mounting a granulamatous response which is the destruction of non activated macrophages which contain bacilli. This leads to an accumulation of dead material known as caseous necrosis. If the activated macrophages are incapable of containing the disease within the resulting lesion, it spreads further in the local tissues, lymph nodes and possibly on throughout the body. At each new site of infection the process repeats itself (Dannenberg and Rook, 1994).

The response to infection with tuberculosis is localised initially because only macrophages around the infected site become activated to a high degree. Overall, the humoral immune system shows little reaction to tuberculosis infection, however, macrophages in the humoral system can become slightly more active. This is thought to be a result of inflammation and small amounts of product released into the circulation from localised lesions (Mackaness, 1964).

The level of macrophage activation may lead to an immune state known as hypersensitivity. Hypersensitivity is the condition occurring when the bacillus is re-encountered and where lymphocytes and macrophages are highly sensitive to the bacillus as a result of the initial encounter. The condition can be either detrimental or beneficial to a host, depending on the number of bacilli presented in the second encounter. If there are a high number, the vigorous response of hypersensitive cells can be seriously damaging to the host. The intensive destruction of infected macrophages extends to include destruction of healthy surrounding tissues. Inflammation due to this activity compounds the problem through reduced local circulation and, possibly, concentration of toxic products released from dead and dying cells (Dannenberg, 1968).

If the number of bacilli is small, hypersensitivity causes the accumulation and multiplication of sensitised macrophages. The effect of this is a stronger defence against bacilli in the area local to their presentation. Generally however, the number of inhaled bacilli which reach the alveolar surface is small, for example between one and three, and there is insufficient tuberculin released to be toxic to macrophages, but enough to elicit a protective response (Dannenberg, 1968).

1.5. VACCINATION

1.5.1 Introduction

Vaccination is defined as the introduction of a prepared vaccine into the body which is strong enough to stimulate the recipient to make antibodies but not strong enough to result in the harmful effects of the disease. The aim of vaccination is to produce a higher than natural state of immunity to a specific disease (Villee *et al.*, 1984). This immunity is referred to as either active or passive. Active immunity results from the active production of antibodies by the subject in response to a vaccine. This type of immunity may be increased by periodic boosters or exposure to the organism. Passive immunity is where the specific antibodies are introduced directly into the subject, requiring no effort on the part of the recipient's body. This method produces immunity more quickly, though it is comparatively short lived, usually lasting only a couple of weeks (Brand *et al.*, 1971). This review will focus on the vaccine used for protection against tuberculosis, which induces active immunity.

Vaccines may be administered by a variety of methods, including orally or by inhalation of an aerosol, by subcutaneous or intradermal injection or infusion into the mammary gland (Blood and Studdert, 1988). The vaccine usually contains a similar but less virulent organism than the one against which protection is offered or contains the disease organism in a harmless state, or contains modified toxins of the organism. The purpose of vaccination is to establish a state of memory immunity. This consists of a long lived population of T lymphocytes that, on recognition of specific antigens can mediate an accelerated recall of previously acquired resistance (Orme, 1999). The problem of how to elicit an effective cell mediated response, as opposed to an antibody response, is still unsolved and is a major focus of current vaccine research (Salyers and Whitt, 1994). Some vaccines can induce either cell mediated or antibody responses depending upon the dose of antigen administered, with low doses favouring the induction of cell mediated immunity. Resistance takes time to establish and it is therefore important that an immunising agent is slow growing and does not reach high levels before a protective response has been established (Bretscher, 1995).

To effectively protect a large population by vaccination it is not necessary to vaccinate every individual. Instead, as the protected proportion of the population increases, a threshold is reached whereby the number of susceptible hosts is too low for the disease to persist within the population

(Buddle *et al.*, 1997). This concept is known as 'herd immunity' and refers to a level of resistance in a population which is sufficient to prevent the entry or persistence of a particular disease (Blood and Studdert, 1988).

1.5.1.1 Rabies: a positive achievement using wildlife vaccination

The epidemiology of rabies (viral disease) in foxes (*Vulpes vulpes*) in Europe is similar to that of tuberculosis in possums in New Zealand. It generally moves in cycles, drops to very low levels when host numbers are low and has a prevalence of 3–7% in areas where rabies is endemic. Vaccination when repeated twice a year in spring and autumn for at least two years successively was proven more effective at controlling rabies than destruction of fox populations by shooting or gassing. Currently 13 countries are involved in the vaccination of foxes against rabies and from 1989 to 1995 rabies incidence has decreased in France by 99%. Complete elimination of rabies has been achieved over large areas resulting in the vaccination program being no longer required, for example Switzerland is now free from rabies as a result of vaccination of foxes (Aubert, 1996), (erson *et al.*, 1981).

1.5.2 Vaccination with BCG

The vaccine against tuberculosis was developed by serial passage of virulent *M. bovis* over ten years by Albert Calmette and Camille Guerin. In 1919, after 230 passages, the vaccine was shown to be avirulent in guinea pigs, cattle and horses. Today, the bacille Calmette-Guerin (BCG) vaccine is in widespread use throughout the world. However the degree of protection afforded by this vaccine is highly variable and has been the subject of many studies. (Bloom, 1994) reports a collection of these with a protective effect ranging from 0–80%. The cause of this variation is the source of much debate and probably includes methodological differences in studies, differences between vaccines, differences in virulence between infectious strains of tuberculosis, BCG protecting against endogenous but not exogenous infection and interference with or masking of protection by environmental mycobacterial infections. Two additional theories are given by Behr, firstly, that variability of protective effect is due to the loss of a substantial number of genes during manipulation of the vaccine over time. The second possibility is that, by becoming accustomed to laboratory culture, BCG strains may have lost the ability to maintain a suitable infection required to develop a strong and long lasting host immune response (Behr and Young, 1999).

The aim of vaccination with BCG is to protect the recipient against contact with fully virulent tuberculosis. The procedure consists of administering live, attenuated bacilli which invade host macrophages and replicate briefly before being killed (Salyers and Whitt, 1994). This replication stimulates the secretion of IgA and IgG immunoglobulins (Wilkie, 1982) which in turn function as part of an effective cell mediated immune response and also memory cells which will allow an accelerated response to the invader, should it ever be encountered again. Bacilli must be alive and actively invade macrophages to ensure successful vaccination. This is demonstrated by vaccination of possums with killed *M. vaccae* inducing no protection against a challenge with virulent *M. bovis* when compared with unvaccinated controls. In contrast, vaccination with live BCG resulted in fewer animals developing lesions and in a reduction in number of lesions in diseased animals (Buddle *et al.*, 1995).

As cattle have been the cornerstone of *M. bovis* persistence over time, it seems natural that a vaccine strategy would primarily target this reservoir of infection. Early trials evaluating the efficacy of BCG began in the 1920s in cattle and used comparatively high doses (1 x 10^7 to 1 x 10^9 colony forming units (cfu)). Results were inconclusive though vaccination did seem to reduce the severity of disease (Francis, 1958). It was suggested that lower doses of BCG may preferentially stimulate the appropriate immune response for protection against mycobacterial infections (Bretscher, 1992). This suggestion is supported by a study which used subcutaneous vaccination with 1 x 10^4 to 1 x 10^6 cfu of BCG in calves. Subjects were significantly protected from the development of tuberculous lesions resulting from experimental challenge (Buddle *et al.*, 1995).

However the situation in New Zealand concerning *M. bovis* and cattle has additional considerations. Immunisation of cattle with BCG results in some animals displaying positive skin test responses to bovine PPD (tuberculin). The PPD method is currently used for identification of tuberculous cattle in New Zealand, and the confusion of results due to vaccination has serious consequences. Primarily it limits the use of tuberculosis vaccines in cattle to those animals which are not destined for the export market. The large scale vaccination of cattle in New Zealand would require international approval and be run in addition to continued wildlife control programs. It would also require a guarantee that there were no risks to human health from vaccinated animals and acceptance by the various international regulatory bodies (Buddle *et al.*, 1997). Regardless of the acceptance and success of vaccination of domestic livestock, it still leaves the problem of tuberculosis in New Zealand's wild life which ensures domestic stock will remain permanently at risk and vaccination continues indefinitely. Alternative options are required, in addition to current control strategies, which address the problem of a wildlife reservoir of tuberculosis, in particular the possum. One of these options is vaccination of possums with BCG.

1.5.2.1 Vaccination of possums with BCG

Several studies have observed the effects of vaccinating possums with BCG by a variety of routes. In general, results have shown the vaccine to have a protective effect (Buddle *et al.*, 2000). An effective vaccine is the best option for the control of the wildlife reservoir of tuberculosis represented by possums in New Zealand (Buddle *et al.*, 2000).

Vaccination of possums with 4 x 10^6 cfu of BCG markedly reduces the severity of disease resulting from challenge with virulent *M. bovis*. However the route of administration has a large effect on the level of protection. The greatest reduction in disease severity was observed when vaccine was administered intratracheally or subcutaneously in comparison with intragastrically vaccinated and non vaccinated animals (Aldwell *et al.*, 1995). Effectiveness of protection was measured by lung weight, lesion presence and type and also by lymphocyte blastogenic responses.

A second study compared the intraduodenal (1 x 10^8 cfu), intragastric (1 x 10^8 cfu) and subcutaneous (1 x 10^6 cfu) routes of vaccination in possums. Intraduodenal vaccination provided the best protection and all vaccinated animals showed better resistance to disease than non vaccinated controls. Protection was measured by body weight change after challenge, change in lung weight, numbers of acid fast bacilli and lymphocyte blastogenic response (Buddle *et al.*, 1997).

A third study compared vaccination of possums with BCG by aerosol (4 x 10^6 cfu), orally (3 x 10^8 cfu) and subcutaneously(1 x 10^6 cfu). Results showed the aerosol and subcutaneous routes to provide the highest levels of protection as measured by minimal change in body and lung weight following challenge with virulent *M. bovis*. However lymphocyte blastogenic responses were low in aerosol and oral vaccinates compared with subcutaneous vaccinates. All routes of vaccination reduced the spread of disease resulting from challenge (Aldwell *et al.*, 1995).

Several computer models have investigated the possibility of eradicating tuberculosis from wild possum populations by vaccination using the herd immunity concept (section 5.1). One model indicates that protection of 54% of the possum population is the threshold at which the disease will be eradicated (Barlow, pers. com., 2000). A second model claims maintenance of 40% of the population in an immune state will eradicate tuberculosis. It also suggests that the prevalence of tuberculosis in possums could be reduced to 10% of its pre-control level within five years by vaccinating possums at a rate of 13% per year (Roberts, 1996).

It is clear that vaccination of possums by a number of routes can reduce the level of disease resulting from infection with *M. bovis* and that some methods appear more effective than others.

However many additional factors must be considered when determining the method best suited to the large scale vaccination of wild possum populations.

1.5.2.2 Oral vaccination

At present, the easiest method of administration would be orally; by baits because parts of this system are already in place as a result of current possum control operations. These control operations currently manufacture and distribute baits over large areas. However, this is an expensive and labour intensive exercise which would probably have to be repeated at least annually (Tyndale-Biscoe, 1991). In addition, because dominant possums may preferentially consume attractive items of food, the application density of oral baits may need to be very high to achieve adequate population coverage. There are also potentially adverse effects of bait consumption by non target species, for example cattle, deer and children.

In addition, there is at present one serious biological flaw in the concept of oral administration of BCG to possums. The oral route is ineffective in possums because of the stomach environment which has a pH of 3–4 (Tyndale-Biscoe, 1973). This causes deactivation of the bacilli before reaching the small intestine, which is their target for eliciting an immune response (Schwarting, 1948). Very high rates of ingestion would be required for sufficient vaccine to remain viable after passing through the stomach. However, intraduodenal vaccination of possums, whereby the stomach is bypassed, has been shown to provide a higher level of protection than any other route (Buddle *et al.,* 1997). This finding is encouragement to persevere with an oral bait.

A potential solution to this problem would be to encapsulate the microorganism in some way. For example, antigens have been administered with a solution of sodium bicarbonate, or packaged in gelatin capsules coated with substances that are insoluble in acidic conditions (Mestecky and McGhee, 1989), releasing their contents in the alkaline environment of the small intestine. The key to releasing the vaccine from the capsule would be the change to an alkaline pH. Unfortunately, the mouth is also a strongly alkaline environment, and would cause premature release of the vaccine from its encapsulation. The release mechanism must be something unique to the small intestine. Production costs of a bait must also be low, because of the volume required for distribution over very large areas and due to the strong possibility that bait distribution will need to be repeated every one to three years.

Oral vaccination has proven effective in the past, in particular against rabies in foxes in Europe and North America (Artois *et al.*, 1997), (Sanderson *et al.*, 1981). However, failures have also been recorded, for example oral vaccination of horses against the bacteria causing strangles failed to induce antibody production (Wallace *et al.*, 1995).

1.5.2.3 Vector transmission

A self transmitting or vector transmitted vaccine is the second potential method of vaccination. The vector would probably be some form of virus or parasite in which genes from *M. bovis* have been inserted by genetic engineering. These genes would encode for antigens which would be recognised by the host. There are two problems with this alternative, firstly, public acceptance of this method would be difficult to obtain on the grounds of the genetic engineering requirements (Bloom, 1994). The second problem would be identification of a possum specific vector and ensuring that it could not cross to other species, particularly humans.

A third possibility is the use of an aerosolized BCG vaccine targeting the respiratory tract of the possum. This avenue is discussed in more depth.

1.6. AEROSOL VACCINATION

1.6.1 Introduction to aerosol vaccination

An aerosol is a solid or liquid particle, suspended in air or a gaseous environment (Blood and Studdert, 1988). Aerosols may contain a wide range of small particles including pathogenic microorganisms. Experimental trials have demonstrated that microbes can be sprayed from, or freeze dried as suspensions in solutions of all main classes of water-soluble or water-compatible materials including sugars, proteins, vitamins, dyes, faeces and saliva (Cox and Wathes, 1995). As the understanding of airborne disease transmission grew, so did interest in the possibility of administering vaccines by the same route (Middlebrook, 1961). The first recorded studies of aerosol vaccination were for Newcastle disease in chickens in 1952 (Hitchner and Reising, 1952), followed by vaccination against distemper virus in mink in 1954 (Gorham *et al.*, 1954). Since then many other species and vaccines have been investigated with respect to aerosol prophylaxis.

1.6.2 The aerosol vaccine

The constituents of an aerosolized vaccine and system by which it is delivered are extremely important. They determine the volume and viability of vaccine reaching the desired site and also the pattern of deposition. This in turn determines the degree of protection afforded by the vaccine (Buddle *et al.*, 1997), (Jadin, 1980). There are three general considerations which must be taken into account when vaccines are dispensed as aerosols. Firstly, the fundamental qualities of the active agent within the vaccine must be retained in an aerosolized form. Secondly, those vaccines known to be allergenic by injection should not be administered by aerosol. Thirdly agents causing hypersensitivity when injected need to be thoroughly tested to ensure the effect is not magnified when administered by aerosol (Walker and Stephen, 1977).

Organisms used in live vaccines administered as aerosols are subject to a reduction in viability. This is due to the stresses of aerosolisation and exposure to the natural environment. The magnitude of this loss is a critical factor when determining the volume of viable vaccine required to generate a protective response. In the case of bacteria, the situation is complicated by the fact that standard culture methods may not indicate true viability after aerosolisation. This is because some environments into which aerosolised bacteria are introduced cause the bacteria to enter a viable, but non-culturable state (VBMC) (Roszak and Colwell, 1987;Heidelberg *et al.*, 1997).

Aspects of the delivery system must also be taken into account, in particular the volume of vaccine to be administered. Spray volume is determined by the concentration of the drug in the spray suspension, air flow rates and the characteristics of the nebulizer. The fact that atomization increases the concentration of a solution must also be considered (Walker and Stephen, 1977).

The lung size and frequency of breathing cycle (respiratory minute volume) of the subject is also an important issue when determining the amount of vaccine which must be inhaled to elicit a suitable immune response (Walker and Stephen, 1977). In any animal population there is a spectrum of immune responses induced by any form of vaccination. Selection of the most appropriate vaccine type and method of administration will minimise the proportion of the population which show little or no response.

1.6.3 Deposition

Aerosol vaccination with a live bacterial vaccine requires that the living microorganism contacts a mucosal surface or the lung after passing into the respiratory tract. The process of attachment involves interactions between the microorganism and host which are primarily influenced by local immunity and the mucous lining of the respiratory tract (Babiuk and Campos, 1993). While these immunmological interactions have already been described (section 4.0), technically there are a number of factors that influence the way in which aerosols are deposited within the respiratory tract. These are the physical qualities of the air ways, deposition forces and hygroscopicity.

1.6.3.1 Physical properties of air ways

The anatomical arrangement and physical dimensions of the subject’s airways in addition to the breathing pattern, affect the rate of deposition by influencing velocity of the air, times of transit of the air from place to place within the system and from moment to moment throughout the breathing cycle (Hatch and Gross, 1964), (Padfield, 1987). From the trachea inwards, the respiratory airways are composed of progressively branching tubes of decreasing size and cross sectional area. At first the airways are simply ducts for air passage, but as their size decreases they also take on a respiratory role (Altiere and Thompson, 1996). There are a gradually increasing number of tubes relative to depth within the respiratory tract resulting in an increasing total cross sectional area as depth into the lung increases. The consequence of increasing cross sectional area is a marked decrease in the velocity of the air as it penetrates further into the lung. Even during gasping breaths, air enters the pulmonary air spaces with a maximum velocity of only a few centimetres per second, resulting in essentially laminar air flow (Reid, 1973).

The last quarter to one third of air volume inhaled during a single breathing cycle (end tidal air) is unlikely to reach the pulmonary air spaces. As a result, fewer suspended particles, especially those 1–6 ?m in diameter, are deposited within the respiratory tract from this air compared with air inspired during the beginning of inhalation (Hatch and Gross, 1964).

1.6.3.2 Deposition forces

The shape of the airways influence the forces which act on inhaled particles. Bacteria and viruses have been shown to exhibit the same aerodynamic behaviour as other organic or inorganic particles when airborne (Cox and Wathes, 1995). The principal forces involved are inertia resulting from the respiratory effort and sedimentation due to gravity and diffusion. Inertia and sedimentation are key factors when particles are greater than 0.5 ?m in diameter, while diffusion becomes the dominant force when aerosols are smaller than this (Knight, 1973;Martonen and Yadong, 1996). Factors affecting the inertia of an aerosol within the respiratory system include the size, density, concentration and shape of the aerosol particle. Deposition is also affected by the solubility and hygroscopicity of a particle (Padfield, 1987). Where particles are in the form of a spray, the concentration and velocity of the spray, in addition to possible propellant evaporation, will also influence the relative importance of the forces involved in particle deposition (Padfield, 1987).

1.6.3.3 Hygroscopicity

Hygroscopicity is the enlargement of small particles (<6 ?m) due to their contact with very humid (saturated) air. It becomes important very deep in the respiratory tract where air in the tertiary bronchioles and alveolar ducts is saturated. The effect of hygroscopicity is to increase the size and mass of small particles. The forces of inertia and sedimentation increase due to the increased mass of the particle, thereby increasing the rate of deposition (Knight, 1973), (Martonen and Katz, 1996). The effect of hygroscopicity is relevant to an aerosol containing *M. bovis* as the bacillus is approximately 5 ?m in length.

Table 1. Deposition of 1.5 ?m hygroscopic particles within the human respiratory tract (Knight, 1973)

Area	% of total air	% deposition (hygroscopic)	% deposition (nonhygroscopic)
Nose	+-6	36*	25
Pharynx to secondary bronchi	10	1**	0
Tertiary bronchi to respiratory bronchioles	21	25**	10
Alveolar ducts	63	21**	13
Total retained		83	48

*24 % of 2 ?m particles retained in inhalation: 12% of total inhaled particles 4 ?m in diameter retained in exhalation due to accretion of water.

**Retention as 4 ?m particles.

1.6.3.4 Studies of aerosol deposition

The patterns of deposition of aerosolized particles have been examined in a number of studies and across a range of species. In general, the results of these studies have found that particles with a diameter of 8 ?m or larger typically penetrate no further than the nasal passages and oropharynx. Optimum pulmonary deposition was achieved when aerosol particles were between 1.5 and 4 ?m (Walker and Stephen, 1977), (Wilkie, 1982). A particle size of 0.5 ?m has the lowest rate of deposition. Below this size, deposition increases due to diffusion, while above it deposition increases due to inertia and other forces mentioned above (see section 6.3.2). During quiet breathing, the rate of deposition due to diffusion and gravity settlement are high, while during heavy breathing, a higher percentage of coarse particles are deposited due to increased inertia as a result of increasing air velocity in the upper respiratory tract (Hatch and Gross, 1964).

Studies of aerosol deposition in chickens showed that particles of 3–7 ?m diameter are captured in the head and anterior trachea. As particle size decreased, there was an increase in depth of particle penetration into the respiratory tract. Due to their tendency to follow streamlines around obstacles, rather than impacting on them, particles 0.5–1.1 ?m were deposited primarily in the lungs (Hayter and Besch, 1974).

The deposition of aerosols in the human respiratory tract has also been thoroughly examined. Particles greater than 6 ?m are typically trapped in the nose while those smaller than 2 ?m reach the lower respiratory tract and alveoli (Knight, 1973). Although larger particles (10 ?m) are consistently filtered in the nose across a range of air-flow rates, increasing flow results in higher levels of small particles (1–2 ?m) also being filtered out (Hatch and Gross, 1964). Most particles smaller than 0.5 ?m remain in the tidal volume and are exhaled (Beech, 1991). Exhaled end tidal air has been shown to be almost devoid of 6 ?m particles and contain less than half the inspired level of 1 ?m particles (Hatch and Gross, 1964), indicating high rates of deposition. A high rate of particle deposition has also been shown in guinea pigs, where one in every three or four inhaled infective droplet nuclei bearing a single bacillus (approximately 5 ?m in size) would reach a susceptible locus (Middlebrook, 1961).

In rats, the response to deposited particles has been shown to vary between nasal, tracheal, bronchial and bronchoalveolar compartments of the respiratory tract (Hensel, 1996). In terms of an aerosol vaccine, if a particular site within the respiratory tract is shown to provide an increased immune response, characteristics of the aerosol delivery could be manipulated to ensure the maximum volume of vaccine is directed at the appropriate site (Wilkie, 1982).

1.6.4 Survival of aerosolised micro-organisms.

The survival rate of any microorganism, including those used as a live vaccine, is a prerequisite for infectivity (Hensel, 1994). Survival rate in the natural environment is adversely affected by a range of environmental factors. The magnitude of the effect varies depending on the organism and becomes greater with decreasing aerosol size. These factors are collectively known as the open air factor (OAF) and defined as the loss of viability due to exposure to open air (Druett, 1973). For most microorganisms the effects include desiccation, exposure to radiation, pollutants and even oxygen, which has been shown to inactivate some gram negative bacteria (Cox and Wathes, 1995). A number of studies have measured the open air factor. (Druett, 1973) found that 1 ?m particles of *E. coli* suffered a 10% loss of viability per minute under normal humidity conditions (60–100%) at night. Survival of Pasteurella multocida was found to be 80% after one minute through a range of humidity levels (Thomson *et al.*, 1992). Under optimum conditions studied (55% relative humidity and 4 degrees celsius), the infectivity of pseudorabies virus in an aerosol was shown to be reduced by 50% in less than one hour (Schoenbaum *et al.*, 1990). Rotavirus demonstrated a similar pattern (Sattar (1984) cited in Schoenbaum *et al.*, (1990)).

In contrast to the above findings, other studies have found that the OAF has minimal impact on viability. For example, Beard and Sanderson (1967) found that two viable strains of mycoplasma (*M. gallisepticum* and *M. meleagridis*) could be recovered from artificially generated aerosols up to 24 hours after suspension. Additionally, in an investigation of the viability and distance traveled by aerosols from slurry spreading equipment, no consistent association was found between the infectivity of viable *Serratia rubidaea* (bacteria) and relative humidity, air temperature or sunlight. (Hahesy *et al.*, 1995).

Survival and retention of antigenic properties after aerosolisation has also been shown in *Streptococcus suis* (bacteria) (Brown *et al.*, 1997). Direct counts and direct viable counts of three other types of bacteria suspended as aerosols (*Serratia marcescens, Klebsiella planticola* and *Cytophaga allerginae*) indicate all three remained viable for at least four hours after aerosolisation (Heidelberg *et al.*, 1997). These findings indicate that there are a number of organisms capable of surviving the stresses of nebulisation, suspension in airborne particles and impingement on a liquid filled air sampler.

It is possible to manipulate the survival characteristics of aerosolized microorganisms to some extent. Addition of serum or glucose, for example, increased the survival rate of *Actinobacillus pleuropneumoniae.* Due to the increased viscosity resulting from these additives, it also enlarged aerosol size from approximately 1 ?m to 4 ?m. Manipulation of temperature and relative humidity has been found to influence the survival of pseudorabies virus (Heidelberg *et al.*, 1997).

1.6.5 Aerosol vaccination in practice

Aerosol vaccination has undergone considerable development since the earliest trials in 1952. Today, it is being used commercially in several fields, primarily against viral disease in commercial poultry production and in the pork industry.

1.6.5.1 Poultry

Aerosol therapy is well suited to intensive poultry systems with high densities of animals in a confined, regulated environment.

There are many studies investigating the effect of aerosol vaccination of poultry against Newcastle disease caused by a viral infection. Full protection was achieved by (Hungerford, 1969), compared with mortality of 10–30% in chickens vaccinated by spray or drinking water (Latif *et al.*, 1981;Ibrahim *et al.*, 1981). Eck (1990) found aerosol exposure twice weekly for two or three weeks gave the best protective effect.

Aerosol protection against the viruses causing fowlpox (Deuter *et al.*, 1991), fowl paralysis (Pridybailo *et al.*, 1986), and infectious laryngotracheitis (Redmann *et al.*, 1983), (Hilbink *et al.*, 187) have also been investigated with encouraging results. Aerosol vaccination of ducklings against the hepatitis virus produced a protective response (Balla and Veress, 1984).

Immunity to *Mycoplasma gallisepticum* (mycoplasma) infection after aerosol vaccination was significantly better than vaccination by intratracheal injection or intranasal instillation, and lasted for at least three months (Hayatsu *et al.*, 1974). (Lin and Kleven, 1984) found aerosol vaccination of chickens to be more effective than eye drop administration for *M. galliscepticum* although the difference was not significant.

Three applications of aerosol vaccine against *Pasteurella multocida* (bacteria) protected 70% of turkeys against challenge with virulent cholera, compared with 7% of controls. One aerosol vaccination followed by one injection provided similar levels of protection (Michael *et al.*, 1986).

Aerosol vaccination against the bacteria causing listeriosis was found to be effective when the immunising dose exceeded 5 x 10^9 bacteria and was repeated after ten days (Eliseeva, 1976).

The stress effect on the respiratory tract in chickens was not influenced by the size of the aerosol across a mean particle size range of 1.0–4.0 ?m (Allan and Borl, 1980).

1.6.5.2 Pigs

Pigs were protected against the virus causing classical swine fever (*Pestivirus*) by aerosol vaccination with either lapinised ‘C’ vaccine or Celvivac vaccine (Popa *et al.*, 1982). In contrast, aerosol and intramuscular vaccination against *Mycoplasma hyopneumoniae* failed to protect pigs against intratracheal challenge (Murphy *et al.*, 1993).

Petzoldt conducted a study in which pigs were vaccinated against the bacteria causing Erysipelas (*Erysipelothrix rhusiopathiae*) using a bacterial concentration of 1 x 10^9/ml. Subjects were exposed for 15 minutes to aerosols of 0.5–5.5 ?m diameter, resulting in the inhalation of 0.13 g of vaccine. Testing immunity two weeks after vaccination resulted in the death of controls, while vaccinated animals survived the challenge of $100LD_{50}$ (Petzoldt *et al.*, 1980).

Six aerosol vaccinations protected pigs against the bacteria *Actinobacillus pleuropneumoniae* when administered to the respiratory or gut mucosae. In comparison, six oral vaccinations offered poor protection. The protective effect of five oral vaccinations was improved when combined with a single dose by aerosol (Nielsen *et al.*, 1990), (Loftager *et al.*, 1993).

A metered dose, propellant driven applicator was designed for delivering *Streptococcus suis* into the respiratory tract of pigs. The device was designed to discharge during the inhalation part of the breathing cycle. It would generate respirable size aerosols (5 ?m) at a concentration of 40 mg/ml and bacterial densities of 0.2, 0.4 and 1.0 x 10^{10}/ml. The increase in both total released bacteria and respirable bacteria were less than proportional to increases in the bacterial densities within the propellant. Testing showed the respirable percentage of bacteria to range from 47% to 67%. This indicates that a large fraction of the bacterial aerosols were clumped into sizes greater than 12 ?m. Those which did not clump were predominantly in the 1–5 ?m range. Additional testing showed that aerosolized bacteria retained their antigenic properties. When using a longer nose cone, which acted as an aerosol expansion chamber, better penetration was achieved. The use of a nose cone has also been shown to improve aerosol penetration in humans (Brown *et al.*, 1997).

1.6.5.3 Cattle

(Mann *et al.*, 1983) found that aerosol vaccination with an attenuated strain of virus causing IBR/IPV (infectious bovine rhinotracheitis and infectious pustular vulvovaginitis) protected calves experimental infection. In contrast, aerosol vaccination of calves with *Pasteurella haemolytica* in (Jericho and Langford, 1982) study failed to provide protection against experimental respiratory disease.

1.6.5.4 Other animals

Foals (*Equs equs*) vaccinated by aerosol against viral respiratory infections showed higher levels of protection compared with a subcutaneous injection of the same vaccine(Zabegina *et al.*, 1999).

Vaccination by both aerosol delivery and intra muscular injection significantly reduced mortality in mink (*Mustela vison*) due to the virus causing distemper (Asztalos *et al.*, 1983). (Scott and Glauberg, 1975) found that domestic cats could be protected against feline panleukopenia virus (FPL) by aerosol vaccination.

Aerosol immunisation of rats before challenge reduced the rate of retention of erysipelas in the respiratory tract. It also caused acceleration of the clearance mechanisms in the upper respiratory tract (Hensel, 1996). Although aerosol vaccination of mice against Aujeszky’s virus provided less protection than intra muscular vaccination, it still reduced disease when compared to controls (Neukirch and Bauer, 1977).

Small aerosols (2.3 ?m) were used to vaccinate the upper respiratory tract of hamsters against infection with *Mycoplasma hyopneumoniae*. As well as providing resistance to subsequent challenge, aerosol vaccination was found to more effectively contain development of the disease when compared to controls (Jemski *et al.*, 1977).

1.6.6 Aerosol vaccination with BCG

Aerosol vaccination with BCG has been shown to induce strong protective responses in a range of species including monkeys (Ribi *et al.*, 1971), mice (Orme and Collins, 1986), guinea pigs and humans (Lagranderie *et al.*, 1996).

Protection with BCG by aerosol vaccination is thought to be due to induction of cell mediated immunity in the lungs, which results in the early activation of alveolar macrophages. The importance of improved local activation of macrophages in tuberculosis infection was earlier emphasised by Dannenberg (1968). This activation is a response to the products of bacilli and products of sensitised lymphocytes being at a higher concentration in the environment local to infection compared to that found systemically (Klein and Horesji, 1997).

(Middlebrook, 1961) found that inhalation of very small numbers of viable BCG organisms by guinea pigs, either as single cells or as clumps can result in a protective response. In this study guinea pigs were vaccinated by aerosol with dose rates of 2.5 x 10^6, 2.5 x 10^5 and 2.5 x 10^4 organisms. After 40 days, all animals in the treatment groups which received either high (2.5 x 10^6) or medium (2.5 x 10^5) doses showed a positive response to tuberculin, while only four of the six subjects in the low dose (2.5 x 10^4) group were found to be tuberculin positive. All controls returned negative responses. Seventy days after challenge with 100 infective units, high dose rate subjects averaged bacterial counts of 0.5 x 10^2 in the lung and 3 x 10^1 in the spleen; medium dose subjects returned counts of 1.7 x 10^2 (lung) and 6.0 x 10^1 (spleen). The control group, by comparison, had bacterial counts of 1.2 x 10^4 (lung) and 1.7 x 10^4 (spleen).

In the same study, aerosol and sub cutaneous vaccination was compared. The aerosol vaccine contained only 20 units of BCG. Twenty eight days after challenge with 200 viable cells, bacterial counts in the lung and spleen of guinea pigs protected by aerosol were 5 x 10^2 and 0.2 x 10^2 respectively. These were significantly lower counts than those obtained from the subcutaneously vaccinated subjects, which had bacterial counts of 30 x 10^2 (lung) and 1.5 x 10^2 (spleen). Immunity resulting from aerosol vaccination was found to persist for at least two years (Middlebrook, 1961). In contrast, two experiments using aerosolized BCG to vaccinate rabbits and three to vaccinate mice failed to show a protective response (Middlebrook, 1961).

Results from an aerosol vaccination trial in guinea pigs by (Lagranderie *et al.*, 1993) suggest that local immunization may prove superior to systemic immunization. It also showed that aerosol vaccination with BCG could restrict the growth of virulent bacilli at the sites of their implantation in the lungs. Similar results were achieved by (Gheorghiu, 1994) who demonstrated higher activation of broncho-alveolar macrophages in animals vaccinated by aerosol in comparison with those vaccinated intradermally.

The possible adverse side effects of high doses of BCG when administered by aerosol have been investigated in guinea pigs. Large doses of BCG (5×10^6 organisms) did not result in the suppuration of the tracheobronchial nodes, or in enlargement of the cervical and hilar lymph nodes. The conclusion was that there are no adverse side effects to the lung or its function (Lagranderie *et al.*, 1993).

A suitable dispenser and method of storage for aerosolised BCG vaccine is a major issue which would need to be addressed before any large scale aerosol vaccination program could be considered. Vaccine dispensers used in the cited studies were complicated, expensive machines, for example the Airborne infection apparatus (Middlebrook, 1961), Turbair Vaccanair apparatus (Latif *et al.*, 1981) and Ultrasonic nebulizers (Lagranderie *et al.*, 1993). These are all impractical for any form of commercial use.

1.6.6.1 Aerosol vaccination of possums with BCG

There is limited information available on the effects of aerosol vaccination with BCG in possums. The most informative study to date is by (Aldwell *et al.*, 1995) who compares the intranasal (aerosol), oral and subcutaneous routes of vaccination. Aerosol vaccination used a dose of 4×10^6 cfu, administered with a simple hand held atomiser nasal pump. The oral dose measured 3×10^8 cfu and the subcutaneous dose rate was 1×10^6 cfu. All routes of administration markedly reduced the severity of disease resulting from challenge with 400 cfu of virulent *M. bovis*. However the protective effect varied between these routes. Protection was measured by weight loss of subjects between challenge and necropsy. Controls, subcutaneous and oral vaccinates all lost an average of 150–760 g. In contrast the aerosol group increased in weight by an average of 60 g. Protection was also measured by granulomas in the liver. When measured in this way, aerosol vaccination provided the best protection. When measured by frequency of granuloma in the spleen, subcutaneous vaccination was most effective, followed by the oral and aerosol route and finally non vaccinates. Overall, the protective effect of vaccine when administered by aerosol was similar to that when administered by the subcutaneous route. In addition, Aldwell notes that aerosol vaccination with BCG may provide more effective protection against a field exposure to *M. bovis* by inhalation, than against an experimentally induced intratracheal challenge. The results of the above study are supported by work in guinea pigs (Lagranderie *et al.*, 1993;Middlebrook, 1961).

The dose of BCG administered by aerosol is difficult to accurately control. However, this may not be critical in the case of the possum as low doses of BCG administered by an appropriate route (sub cutaneous or respiratory tract) have induced protective responses in other animal species (Buddle *et al.*, 1995).

1.7. CONCLUSION

Tuberculosis caused by the bacterium *Mycobacterium bovis* was probably introduced into New Zealand during importation of cattle in the nineteenth century. Over the following thirty years it became a serious public health problem, with infection in humans occurring mainly through the consumption of milk contaminated with *M. bovis*. Today tuberculosis is found in several species of wildlife and a small percentage of domestic cattle and deer herds. However, numerous vector control operations, herd testing and movement restrictions on infected herds have failed to stop the geographic spread of this disease.

The brushtail possum (*Trichosurus vulpecula*) was introduced into New Zealand in the late 1800s to initiate a fur trade. The possum is a very adaptable animal and quickly spread throughout 90% of the country, despite various control efforts. In 2000 the possum was regarded as the nation's major pest species with a population estimate of 70 million. Feeding habits of the possum cause serious damage to native flora and fauna.

The first possum infected with tuberculosis was found in 1969 and today infected possums are found over one third of New Zealand. Since this first discovery, it has become clear that the possum functions as a wildlife reservoir for tuberculosis, acting as a source of re-infection into domestic cattle and deer herds and greatly frustrating disease eradication efforts. Tuberculous possums are also a source of infection for several other wildlife species.

Traditional methods of possum control are based on the large scale aerial distribution of oral baits containing poison (compound 1080), followed up with ground based poison and trapping operations. These methods have failed to effectively control either the spread of the possum or the spread of tuberculosis. There is also increasing public concern over the detrimental effects of large scale poisoning operations on the environment and non target species.

An alternative method for control which may greatly assist in the control and eradication of tuberculosis is vaccination of the wild possum population with BCG vaccine. The vaccination of wild foxes against rabies using oral baits has been successful in Europe. Possums respond well to vaccination with BCG by developing an improved immune response to virulent *M. bovis*. If successful, such a method may be adapted for use in Great Britain and Ireland where a similar problem exists with the badger (*Meles meles*) functioning as a wildlife reservoir for tuberculosis.

Currently, there are three major challenges facing the development of an effective vaccination program. Firstly the viability of a live vaccine must be further researched. Secondly, a commercial product to contain BCG and dispense it as an aerosol must be developed. Thirdly, an efficient and cost effective method of vaccine delivery must be devised. The third challenge is addressed in chapter three.

Chapter 2

A BEHAVIOURAL STUDY OF BRUSHTAIL POSSUMS (*TRICHOSURUS VULPECULA*) WITH CLINICAL TUBERCULOSIS (*MYCOBACTERIUM BOVIS*)

A naturally formed hollow in a clay bank, sheltered by gorse and flax, forms a typical possum den. Den number, possum identification number and dates of use are recorded on the yellow tag.

2.1. INTRODUCTION

The brushtail possum (*Trichosurus vulpecula*) was introduced to New Zealand to start a fur trade during the late 1800s. This highly adaptable animal rapidly spread throughout the country and by the 1960s the population had reached natural peaks in most areas. An estimated 70 million possums now inhabit virtually the length and breadth of New Zealand (Montague, 2000).

Bovine tuberculosis, caused by *Mycobacterium bovis*, was probably introduced to New Zealand in about 1840 with imported cattle. In 1999/2000 about two percent of cattle and deer herds were infected with the disease (Animal Health Board Annual Report, 2000). Tuberculosis was first discovered in the wild possum population in 1967 (ONeil and Pharo, 1995) and it is now considered endemic in this species over approximately one quarter of New Zealand.

In New Zealand the test and slaughter scheme has failed to eradicate the disease from livestock in areas with tuberculous possums. Possum control operations have greatly reduced the level of disease in livestock in the same area. It is now widely accepted that the wild possum population is a reservoir for the disease, acting as a source of infection to livestock and other wildlife species. Wildlife reservoirs of tuberculosis have also been identified in other countries including the badger (*Meles meles*) in Ireland and Great Britain, the Swamp buffalo (*Bubalus bubalis*) in Australia and the white tailed deer (*Odocoileus virginianus*) in the USA.

The route by which disease is transmitted from an infected possum to other possums and livestock is not well understood. Probable routes of transmission include by aerosol or ingestion, though in these species the pattern of disease indicates aerosol transmission is predominant (Jackson *et al.*, 1995, Costello *et al.*, 1998).

The tuberculous possum is most infectious during the terminal stages of disease (Jackson *et al.*, 1995). Possums that are terminally ill with tuberculosis become active in daylight, are disoriented, uncoordinated and do not show normal avoidance reactions (Paterson and Morris, 1995). Both cattle and deer have been shown to actively investigate possums behaving in this way (Sauter and Morris, 1995).

The habitat where transmission most frequently occurs has been the subject of some debate. Paterson *et al.*, (1995) found that tuberculous possums were more likely to transmit the disease to other possums and also domestic stock when in the vicinity of their denning range in scrub. Most possums den in small areas and tuberculous possums remained closer to these areas than healthy possums. Denning areas of tuberculous possums are high risk locations for disease transmission.

Pasture is another possible location for transmission of disease from possums to livestock. This habitat is an important food source for possums where adjacent to bush areas (Green and Coleman, 1986). Interactions between possums feeding on pasture are infrequent and of low intensity (Paterson *et al.*, 1995). Pasture is distinct from other grass areas found within bush areas. It is a specific mix of nutritious species of high palatability to livestock. It is common for pasture to receive artificial fertilisation and be managed to maintain feed palatability and a high growth rate. Management of pasture is distinct from that of grass areas found under scrub and in clearings within the bush.

This observational study investigated the denning and ranging behaviour of possums from the development of clinical signs of tuberculosis until death. The aim of the study was to identify aspects of behaviour of tuberculous possums that may influence transmission of the disease to livestock or healthy possums. The study site was first used to study possum populations with endemic tuberculosis in 1989 as reported by Pfeiffer, (1994). The longitudinal study initiated by Pfeiffer continued until 2000.

2.2. MATERIALS AND METHODS

2.2.1 Study site

The study was carried out between March 1998 and February 2000. The study site was located near Castlepoint (40? 51’ S, 176? 14’ E) in the Wairarapa on the east coast of the North Island, New Zealand. It consisted of 56 ha of mainly dense manuka (*Leptospermum scoparium*) and gorse (*Ulex europeus*) with pockets of flax (*Phormium tenax*), broadleaf forest remnants and groups of ponga (*Cyathea dealbata*). There were also areas of open savannah woodland and pasture. Elevation varied from 60 to 270 metres above sea level. The site was drained by four water ways which were dry during the summer.

Figure 1. Aerial photograph of approximately two thirds of the Castlepoint study site

On the site were 450 cage traps set in fixed locations and divided into three trap lines (appendix 1.1). They were used in a longitudinal study of the resident possum population based on a capture-tag-recapture program. Traps were set at bimonthly intervals for three consecutive nights and baited with slices of apple dusted with cinnamon. At each trapping session all trapped possums were sedated with 100 mg of ketamine (Parnell Laboratories New Zealand Ltd., New Zealand) and weighed. Age was estimated by the degree of wear of the upper first molar on a scale of 1 (unused, characteristic of a dependent joey) to 7 (worn completely flat, characteristic of old age). Condition score was recorded on a scale of 1 (emaciated) to 5 (fat) and for mature females their reproductive status determined from an examination of their pouch and mammary glands. The trap number where the possum was trapped was also recorded.

2.2.1.1 Depopulation of the study site

The longitudinal study at Castlepoint began in 1989. All possums on the site were depopulated in March 2000 over four weeks of intensive trapping. During the depopulation, all possums, including the radio collared possums, were captured in live traps or leg hold traps. Possums were euthanased by intraperitoneal injection of 1.5 ml sodium pentobarbitone (Pentobarb 300, 300 mg/ml, South Island Chemicals Ltd., Christchurch, New Zealand) and were subject to an examination for macroscopic lesions *post mortem*, bacteriological examination for *M. bovis* and histopathology.

2.2.2 Radio collars

Radio collars were fitted to thirty possums during the study. These possums constituted three groups: naturally infected with tuberculosis (14 possums), experimentally infected (eight possums) with tuberculosis, and healthy possums (eight possums).

2.2.2.1 Naturally infected possums

Tuberculosis in naturally infected possums was diagnosed by palpation for swelling of the mandibular, deep axillary, superficial axillary and inguinal lymph nodes during the bimonthly trapping. Isolation of *M. bovis* from culture of swabs taken from draining sinuses or from aspirates of swollen nodes was used to confirm the diagnosis. Radio collars were fitted when clinical disease was detected.

2.2.2.2 Healthy possums

Radio collars were applied to healthy possums to obtain control data. Healthy possums were matched on three characteristics with naturally tuberculous possums where possible. These characteristics were being resident on the same part of the site and matched for age and sex where possible.

2.2.2.3 Experimentally infected possums

During the study experimentally infected possums were released on three occasions. On each of these occasions one mature male was selected from each trap line. They were anaesthetised with

12 mg of Saffan (Pet Elite Ltd, Lower Hutt, New Zealand) and infected with virulent *M. bovis* via an intra-tracheal cannula into the lungs (Pfeffer et al 1995). A radio collar was applied to each possum before they were released back on to the study site. Experimentally infected possums were released in August 1998 (3 possums), February 1999 (3 possums) and August 1999 (2 possums).

2.2.2.4 Possum behaviour

The behaviour of each possum was subjectively assessed as it left the den site during each radio tracking event. Aspects of the behaviour of interest included the ease and speed with which the possum moved and the coordination of movements. Behaviour was used to judge whether a sick possum should be euthanased. Moribund animals were caught and euthanased by intraperitoneal injection of 1.5 ml sodium pentobarbitone. Possums found dead or euthanased were recovered for *post mortem* examination.

2.2.2.5 Den characteristics

Fourteen specific attributes of each den and den site were recorded on a specifically developed form (appendix III). These included height above ground level, degree of exposure of the den to wind, roof material, floor material, the number and size of entrances, and the predominant vegetation type. The condition of the floor was divided into four categories ranging from dry to saturated. The slope and aspect of the immediate area were also categorised. Each den was permanently identified using a plastic cattle ear tag. Den number, date and the identity of the possum were recorded on the tag. The weather conditions, based on three categories of temperature and precipitation, were recorded for the day, previous night and previous day.

The study site was divided into three areas representing areas of high, medium and low density of den sites (appendix 1.2). These areas were used to evaluate the effect of den site density on the denning range of individual possums.

2.2.3 Radio tracking procedure

2.2.3.1 Radio equipment

Radio collars were two stage units that transmitted 60 pulses per minute, powered by 3-3.5 volt battery, had an external aerial and weighed 25 grams (SirTrack, Havelock North, New Zealand). The reception range of the transmitters was approximately 10km within a line of sight although in the rugged terrain of the study site this range was approximately 1km. Battery life span was approximately 15 months. Tracking equipment consisted of a Merlin 12 receiver and yagi aerial as detailed by Paterson (1993).

Figure 2. Radio tracking equipment

2.2.3.2 Radio tracking method

Possums were tracked to their den site weekly. Each den site was examined visually and the characteristics listed above (section 2.2.5) recorded. The den site was related to the nearest trap by distance (metres) and compass bearing. Den sites outside the boundaries of the study site were included.

2.2.3.3 Trapping data

The data on where possums were trapped at the bimonthly trapping events was used to supplement the denning data for each radio tracked possum. The data consisted of the trap number and date. Data was collected between November 1994 and February 2000.

2.2.4 Geographical position data

The coordinates (latitude and longitude) of all trap positions, the perimeter of the study site, the major water course and fence lines were determined using Trimble Global Positioning System (GPS) equipment (Trimble Navigation Limited, Mapping and GIS Sytems, Sunnyvale, CA, USA). The equipment consists of a Base Station™ (satellite detector, GPS computer, radio and antenna) and a roving Asset Surveyor™ (satellite detector, GPS computer, radio and antenna). The coordinates for the base station were determined by averaging 100 readings for latitude, longitude and altitude.

The coordinates of each point were determined using the rover unit. Point coordinates were generated by combining the position of the rover unit with the known fixed position of the base station and satellite information through differential processing. By using this method, points could be defined with more speed and accuracy than with the rover unit alone. Where overhead vegetation prevented a trap position being determined with the GPS equipment, the distance and compass bearing from the nearest trap with a known position was recorded, and the coordinates plotted using a computer program.

The software package Pathfinder Office GeII (Trimble Navigation Limited) was used to down load data from the rover unit and also provided viewing and basic analytical tools. With the traps visible in Pathfinder and units of decimal degrees, the compass bearing and measuring tool were used to manually generate a table of den site coordinates.

2.2.5 Data analysis

Microsoft Access (Microsoft®, Access97) was used for data storage and manipulation. ArcView, version 3.1 (©1996 Environmental Systems Research Institute, Inc.) was used to visualise the data and calculate possum ranges using the extension ‘spatialtools’. This extension was obtained from the Alaska Biological Science Centre (www.absc.usgs.gov./glba/gistools/index.htm.). Animation of the movement between den sites was made possible with the extension ‘AnimalMovement2’ also obtained from this site, allowing the pattern of den use over time to be visualised. The script ‘Coordinate Precision’ was used to identify point coordinates with maximum accuracy (eight decimal places) in ArcView.

2.2.5.1 The kernel density estimator

The denning range, foraging range and activity range for each possum and tuberculosis hotspots (section 2.5.6.) were defined using a kernel density estimator in ArcView. This technique provided a visual representation of the range for both individuals and groups of possums. It also took into account the frequency of den or trap use.

Contours were used to divide the range of a possum into four areas, based on intensity of use. Range size was represented by the area within the outermost contour (area containing 80% of possum locations). The innermost contour encloses the area used with highest intensity. This area contains the central most 20% of possum locations. Each successive outer contour (40%, 60% and 80%) represents a progressively wider geographic spread and lower density of locations. The size of the range was calculated using the convex polygon function in ArcView.

2.2.5.2 Definition of denning range

The denning range was defined as the area that contained 80% of all denning events for an individual possum. The denning range of some possums consisted of two or more distinct areas and in these cases the overall size was calculated by summing the individual areas.

2.2.5.3 Definition of a foraging range

The foraging range was defined as the area that contained 80% of the trapping events for an individual possum.

2.2.5.4 Definition of activity range

The activity range was defined as the area that contained 80% of combined denning events and trapping events for an individual possum. The concept of activity was derived from the ‘core range’ estimate as used by Brockie *et al.* (1987).

2.2.5.5 Definition of a total range

The total range was the area that contained all denning events and trapping events for an individual possum and was derived from the estimate used to identify long forays by Brockie *et al.* (1987). The perimeter of this range was drawn to pass halfway between the outermost trap where a possum was caught and the next trap beyond it, away from the centre of the area. Where there was no trap beyond the outermost trap, the perimeter of the area was arbitrarily set at 50 metres beyond the outermost used trap.

2.2.5.6 Definition of a long distance foray

Long distance forays were identified by both denning and trap data. A long distance foray was defined as a movement, of a distance at least three times the radius of the activity range, from which the possum returns to its established activity range. The minimum distance of a long foray equates to 200 m. These forays do not include dispersing movements as only adult possums were studied.

2.2.5.7 Definition of a hotspot

There were two types of hotspots. Major hotspots consisted of a cluster of dens used by one or more tuberculous possums and were defined as the area in which the likelihood of finding a tuberculous possum in a den was ?60%. Minor hotspots were smaller clusters of dens and defined as areas where the likelihood of finding a tuberculous possum in a den was ?20% and ?60%.

2.2.5.8 Statistical analysis

All statistical analyses were done using SPSS 8 (copyright, SPSS Inc., 1999). Distributions were checked for normality using the confidence interval technique cited in Cramer (1998). The Mann-Whitney U test was used to compare the size of possum ranges. Pearson's correlation was used to examine correlation between number of observations and range sizes. The duration of survival of each possum group was compared using the Kaplan-Maier survival curve. Graphs were produced in Excel (Microsoft, Redmond, USA).

2.3. RESULTS

Denning and survival data was collected for 30 possums: 14 naturally infected, eight experimentally infected and eight healthy possums. Radio collars were applied to 16 possums in 1998 and 14 in 1999.

2.3.1 Radio tracking results

During the study there were a total of 315 tracking events of which 204 were of tuberculous possums and 111 of healthy possums. A total of 233 dens were identified and on 63 occasions possums were using previously identified den sites. There was considerable variation in the denning behaviour of individual possums. On average each possum was tracked to a den 11 times, with a range from one to 56. On one occasion a possum moved before the den site could be identified. Of the 22 tuberculous possums tracked, the carcasses of 17 were found.

Table 2. Survival duration of naturally infected, experimentally infected and healthy possums (weeks)

Possum group	n[1]	Median	Range
Naturally tuberculous	14	11	1-84
Experimentally infected with tuberculosis	8	10	9-21
Healthy	8	26	8-38

[1] n = number of possums in each group

A Kaplan-Maier survival plot (Figure 3) shows the variability in life span after radio tracking began. The median survival time for healthy possums was 26 weeks, for naturally infected possums 11 weeks and for experimentally infected possums 10 weeks.

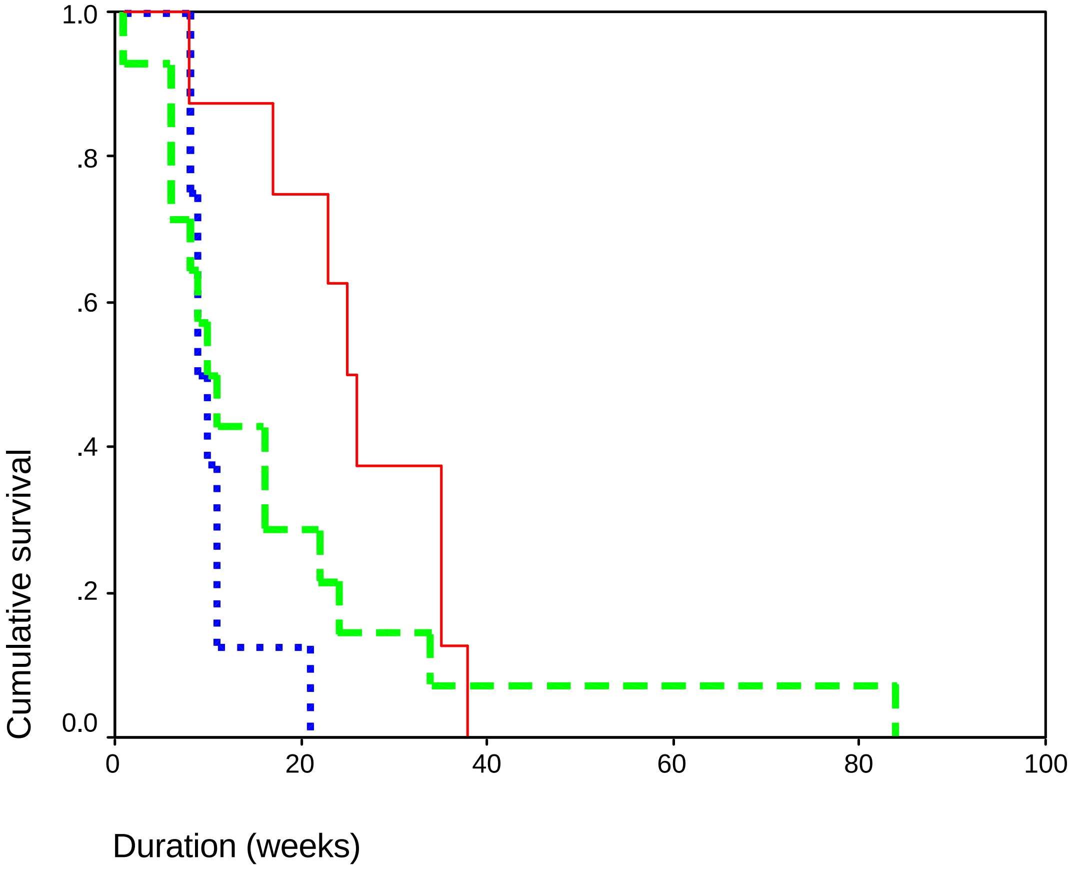


Figure 3. Survival of healthy, naturally infected and experimentally infected tuberculous possums, solid line = healthy possums, coarse dashed line = possums naturally infected with tuberculosis, fine dashed line = possums experimentally infected with tuberculosis

2.3.1.1 Survival duration of radio tracked possums

The measured duration of survival within the study period for individual possums ranged from one week to 84 weeks. The distribution of survival periods for all possums is shown in **Figure 4**. The distribution of survival periods was skewed to the left. The median survival period for naturally tuberculous possums after first being diagnosed with clinical tuberculosis was 11 weeks (Table 2), one week longer than for experimentally infected possums. The survival of naturally tuberculous possums was significantly shorter than healthy possums (26 weeks) (Mann-Whitney U 2.35, z=-2.223, P=0.024). The survival of experimentally infected possums was also shorter than healthy possums (Mann-Whitney U=8.0, Z=-2.534, P=0.010). The shortest survival time was 1 week (Possum 5644, first identified in the terminal stages of disease) and the longest survival was 84 weeks (Possum 5787). Possum 5787 was found with tuberculous lesions in August 1997, and was fitted with a radio collar in March 1998, at the beginning of the study and euthanased during the depopulation. Infection was confirmed when *M. bovis* was isolated from aspirates on two separate occasions but at *post mortem* examination no lesions were detected and *M. bovis* was not isolated from tissue samples collected.

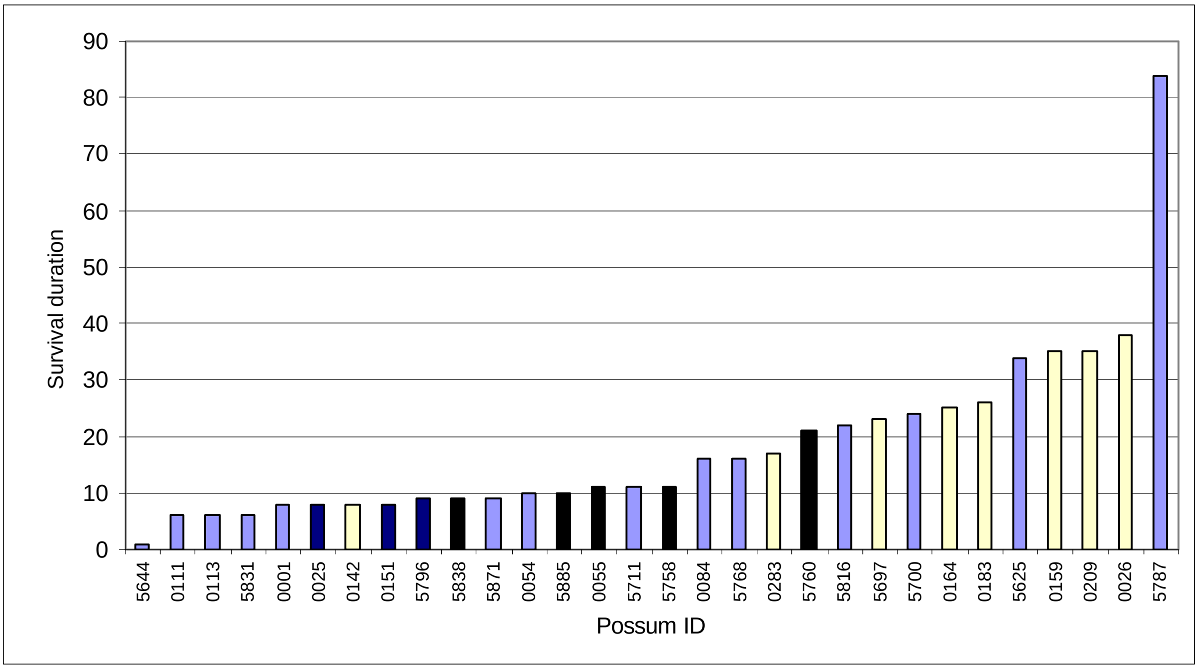


Figure 4. Distribution of survival duration of healthy, naturally infected and experimentally infected tuberculous possums. Blue bars = possums naturally infected with tuberculosis, black bars = possums experimentally infected with tuberculosis, light bars = healthy possums

2.3.1.2 Cause of death of radio tracked possums

Possums died from a variety of causes (Table 3). Most died of tuberculosis, either naturally or were euthanased *in extremis*. A neighbouring farmer trapped four possums within 200 m of the northern boundary of the study site. One tuberculous possum was lost due to failure of the radio transmitter in the collar.

The carcasses of 24 possums were examined *post mortem* including 15 tuberculous possums (Table 3). The carcasses of three possums were very decomposed and were not recovered. One tuberculous female found dead had a small joey, approximately six months old. Gross lesions of tuberculosis were found in the joey at *post mortem* and were indicative of pseudo-vertical transmission.

The radio collar on one healthy possum caused an abscess to form and was removed after five months. This possum was classified as lost to follow up. Due to the failure of a transmitter on one radio collar, possum 5885 was also lost to follow up.

Table 3. Cause of death of radio tracked possums

Cause of death	N[1]	Tuberculous possums	Healthy possums	*Post mortem* examination
Died due to tuberculosis	13	13	0	10
Euthanased *in extremis*	4	4	0	4
Euthanased during depopulation.	5	1	4	5
Trapped by neighbour	4	2	2	3
Killed by bulldozer	1	0	1	0
Died during heart bleeding	1	1	0	1
Lost to follow up	2	1	1	N/A
Total	30	22	8	24

[1] n = number of possums

The distribution of deaths by month for 24 radio tracked possums are shown in Figure 5. The death of experimentally infected possums caused the peaks in October and November 1998 and April and October 1999.

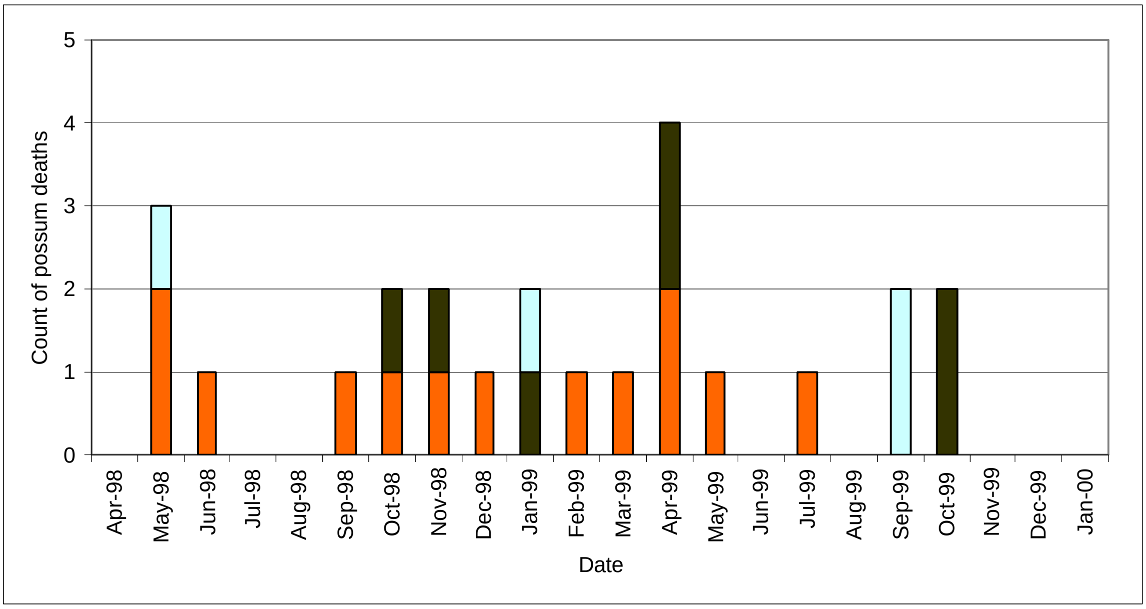


Figure 5. Distribution of deaths for radio tracked possums. Blue bars = healthy possums, orange bars = possums naturally infected with tuberculosis, black bars = possums experimentally infected with tuberculosis. This does not include the one tuberculous and four healthy possums euthanased during the depopulation

2.3.1.3 Behaviour of radio tracked possums

Tuberculous possums showed a range of behavioural states. These were from behaviour associated with healthy possums through increasing debilitation to the final stage where they were moribund and unable to move. For most of the time, the behaviour of diseased possums was indistinguishable from that of healthy possums. The level of physical debilitation increased markedly during the terminal stages of disease, which lasted between one and three weeks. The possums at this stage were dull, lethargic, and showed poor coordination and balance. They were much less responsive to disturbance caused by the tracker. They were unable to climb steep inclines and would frequently fall over. These possums were emaciated and often had visibly enlarged superficial lymph nodes, some of which had burst to form draining sinuses.

2.3.1.4 Location of tuberculous possum carcasses

The carcasses of 17 tuberculous possums were recovered. Thirteen had died and four were euthanased *in extremis* (Table 4). No possum carcasses were recovered from within a den. Two were found on pasture. Three were found in long grass amongst sparse scrub. The remaining 12 possums were found in dense scrub. Two possums were found dead in pools of water in creeks, having apparently drowned, and a third was found next to a creek, having been drowned when the creek flooded. All three were found where the waterways ran through scrub.

The denning range was known for 12 of the 17 tuberculous possums for which the carcass was recovered (Table 4). Six possums were found within their denning range and six were found outside.

The activity range was known for all 17 possums. Eight died within their normal activity range. Nine died outside this area. Of these nine, three possums had moved more than 200 m from their activity range, and six had moved less than this distance.

Ten of the 17 recovered carcasses were found at the lowest recorded elevation point on their activity area.

Table 4. Recovery of carcasses of tuberculous possums: individual possum details, location of carcass, and details of habitat where the carcass was located

Possum number	Source of Tb	Sex	Within den range	Within activity range	Low point (1)	Distance from centre of activity range (m)(2)	Description of site of death
0001	Natural	F	------	yes	no	40	Scrub
0025	Exp. inf	M	yes	no	no	190	Long grass under scrub
0084	Natural	F	yes	no	yes	**230**	Creek in scrub
0111	Exp. inf	M	------	yes	yes	105	Creek in scrub
0151	Exp. inf	M	yes	no	yes	**220**	Scrub
5625	Natural	F	yes	yes	yes	15	Scrub
5644	Natural	M	------	no	no	150	Long grass under scrub
5700	Natural	M	yes	yes	yes	0	Scrub
5711	Natural	M	------	yes	no	25	Scrub
5758	Exp. inf	M	no	no	yes	150	Pasture
5760	Exp. inf	M	no	no	yes	160	Scrub
5768	Natural	F	no	yes	yes	90	Scrub
5796	Exp. inf	M	no	no	yes	170	Pasture
5816	Natural	F	yes	yes	no	20	Scrub
5831	Natural	F	no	no	yes	120	Creek in scrub
5838	Exp. inf	M	no	no	no	**260**	Long grass
5871	Natural	F	------	yes	no	0	Scrub

Exp. inf = possums experimentally infected with tuberculosis

[1] Low point = possum carcass found at lowest recorded elevation

[2] Movements shown in bold were defined as long distance forays (>200 m)

2.3.2 Denning behaviour of possums

Radio tracking identified a total of 233 individual den sites and the distribution of these is presented in **Figure 6**. The preferred denning area for most possums covered 15 ha, or approximately 25% of the area of the site. This area contained the steepest slopes on the site. It was covered with scrub and native forest pockets and was known as Ponga Gully.

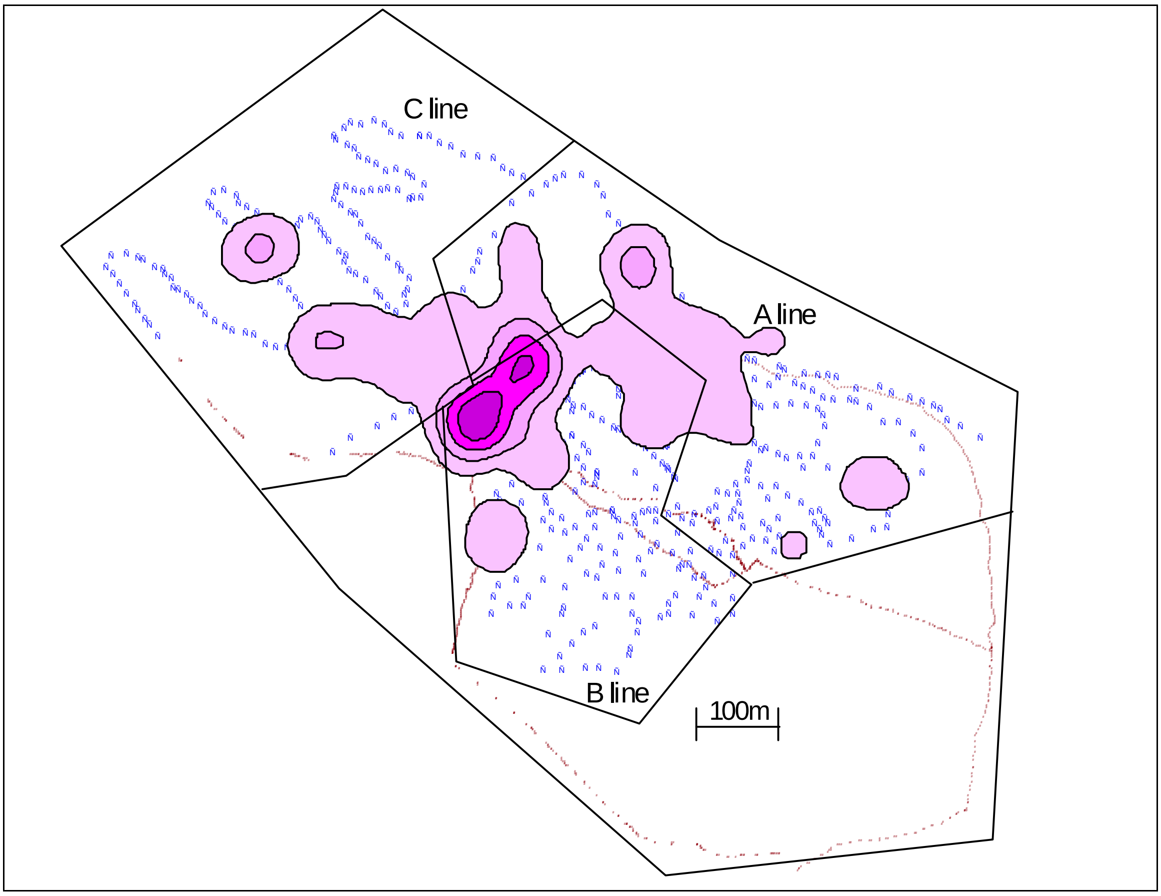


Figure 6. Density of den site use for all radio tracked possums. Crosses represent trap sites, and lines divide the study site into three trap lines, A, B and C. Contours represent den site density, from high (central most 20% of den sites, darkest shade), through 40%, 60%, to low (80% of den sites, lightest shade)

The distribution of den sites used by naturally tuberculous possums (**Figure 7**) was similar to that for all possums. However there were three distinct pockets of dens used by this group. These small foci are indicated as areas 1, 2 and 3 in **Figure 7**. The den sites of healthy possums were grouped into two foci, one in Ponga Gully and a second at the southern end of the C trap line (see appendix 1.8). Den sites of experimentally infected possums were spread more evenly across the study site (see appendix 1.11), indicating that the selection of those possums to provide site-wide coverage was successful.

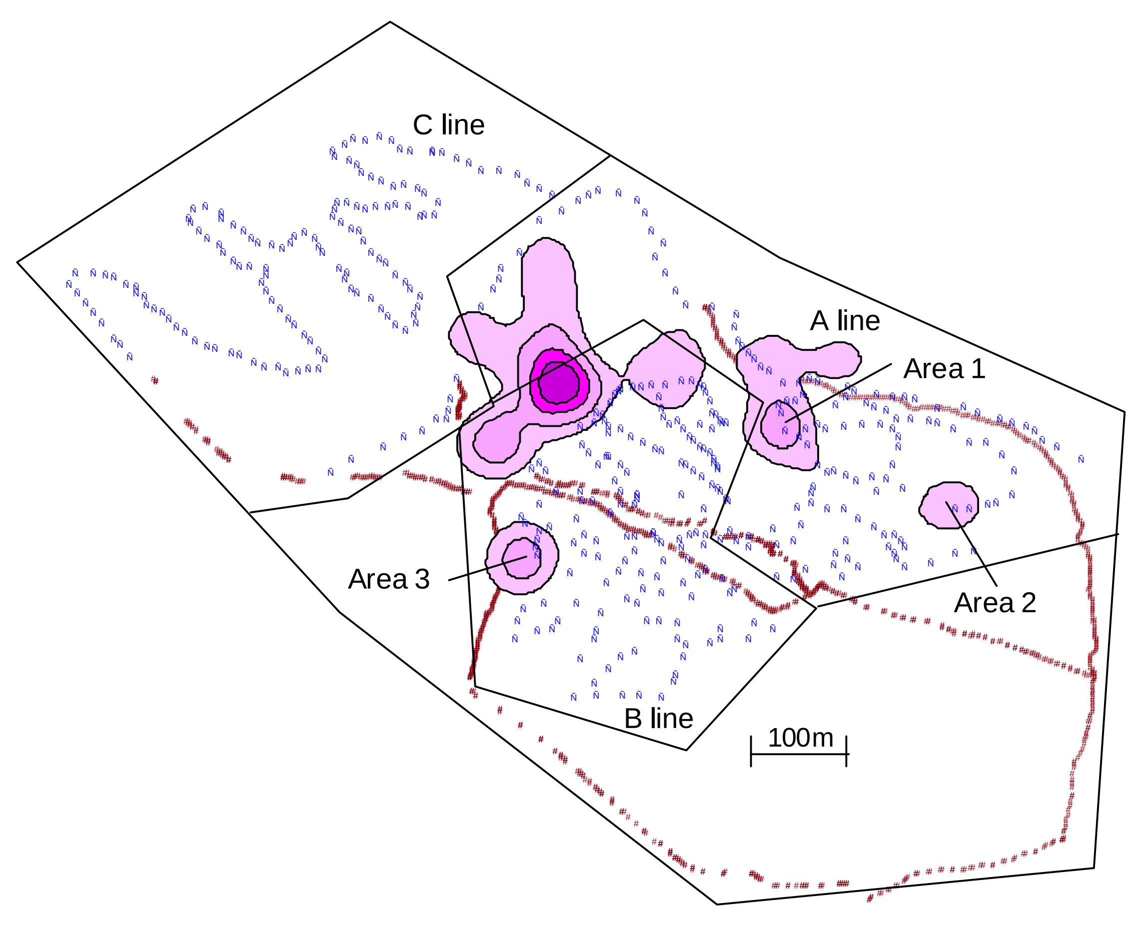


Figure 7. Density of den sites used by naturally tuberculous possums. Lines represent areas covered by the three trap lines (lines A, B and C). Crosses represent trap sites. The three distinct pockets of dens used specifically by tuberculous possums are indicated as areas 1, 2 and 3. Contours represent den site density, from high (central most 20% of den sites, darkest shade), through 40%, 60%, to low (80% of den sites, lightest shade)

2.3.2.1 Denning range for possum groups

There was sufficient data to calculate denning ranges for 22 of 30 radio tracked possums (Table 5). The denning range covered approximately 20% of a possum's total range. There were no significant differences between denning ranges of the three groups of possums.

Table 5. Denning range (hectares) for the three groups of possums: naturally infected, experimentally infected and healthy.

Group	n [1]	Minimum	Maximum	Mean	SD
Healthy	6	0.5	2.4	1.4	0.6
Naturally tuberculous possums	8	0.5	3.1	1.4	0.8
Experimentally infected	8	06	2.1	1.3	0.5

[1] n = number of possums in each group

The size of possum denning range was compared across three den site densities (appendix 1.2). The central portion of the site had the highest density of dens and there the average denning range was 1.2 ha. The eastern part of the site had a medium density of den sites with an average denning range of 1.4 ha. The lowest density of den sites was on the western part of the study site and had an average denning range of 1.5 ha.

2.3.2.2 Denning behaviour of individual possums

Denning ranges were generated for the 22 possums that were tracked at least four times. Denning ranges for possums formed four broad types: a single group of dens (unimodal), two distinct and adjoining groups of dens (bimodal), three distinct and adjoining groups of dens (trimodal) and dens not showing any grouping. The distribution of different denning range types across the three groups of possum is shown in Table 6. Most possums used den sites clustered in a single group (10/22) or two distinct groups (8/22). Seventeen of 22 possums had one or more dens that lay outside the perimeter of the denning range as described above.

Table 6. Distribution of denning range types for the three possum groups: naturally infected, experimentally infected and healthy possums

Possum group	n [1]	Unimodal	Bimodal	Trimodal	Dispersed
Healthy (n=6)	6	1	4	1	0
Naturally tuberculous (n=8)	8	4	2	0	2
Experimentally infected (n=8)	8	5	2	1	0
Total (n=22)	22	10	8	2	2

[1] n = number of possums in each group

The denning range of Possum 5760, which covered 1.3 ha, is an example of a unimodal range (Figure 8).

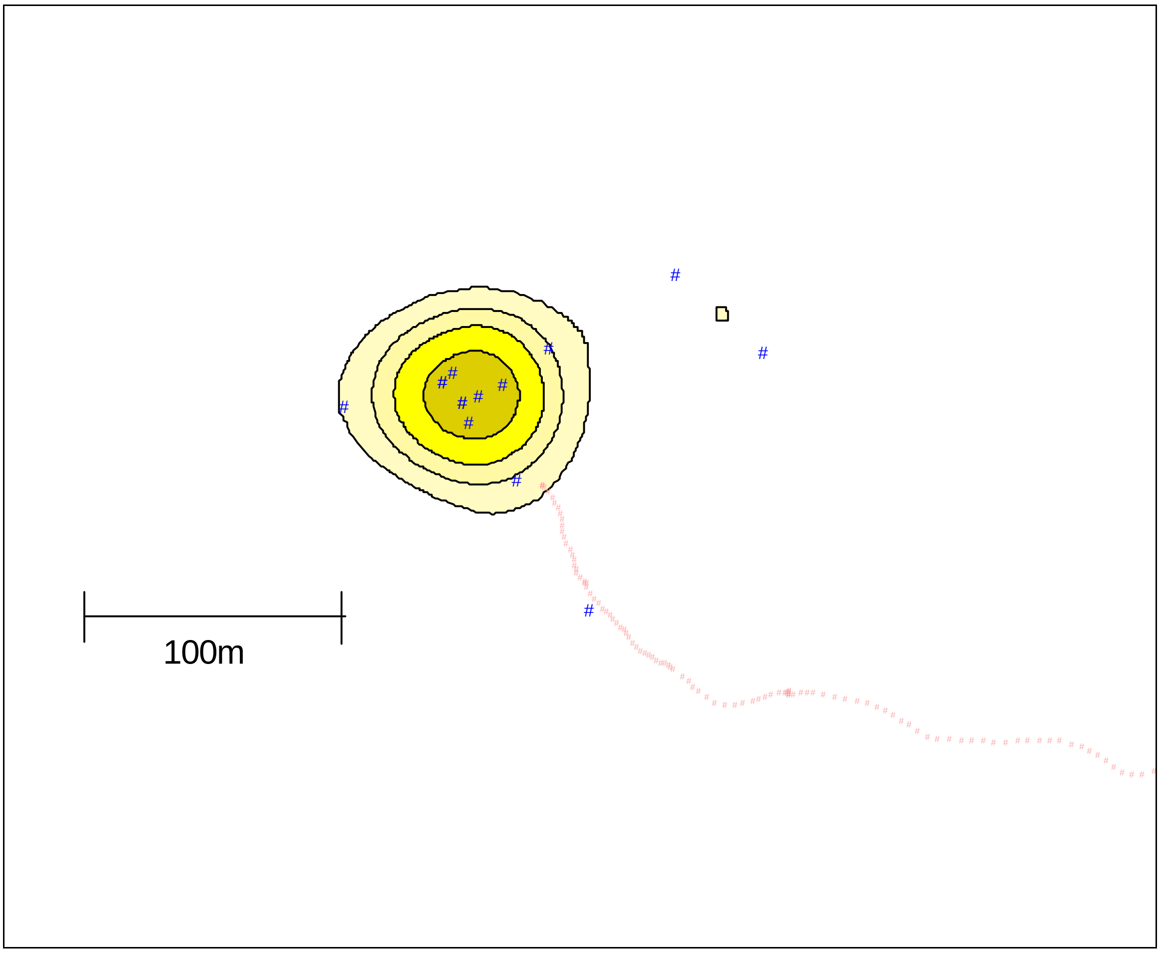


Figure 8. The denning range of Possums 5760: An example of single cluster of dens. Contours represent den site density, from high (central most 20% of den sites, darkest shade), through 40%, 60%, to low (80% of den sites, lightest shade). Den locations are marked by blue dots and the dotted line represents an access track. Note the dens that lie outside the denning range

An example of a bimodal range is illustrated in Figure 9 by Possum 5700, the combined area of the two foci is 0.9 ha.

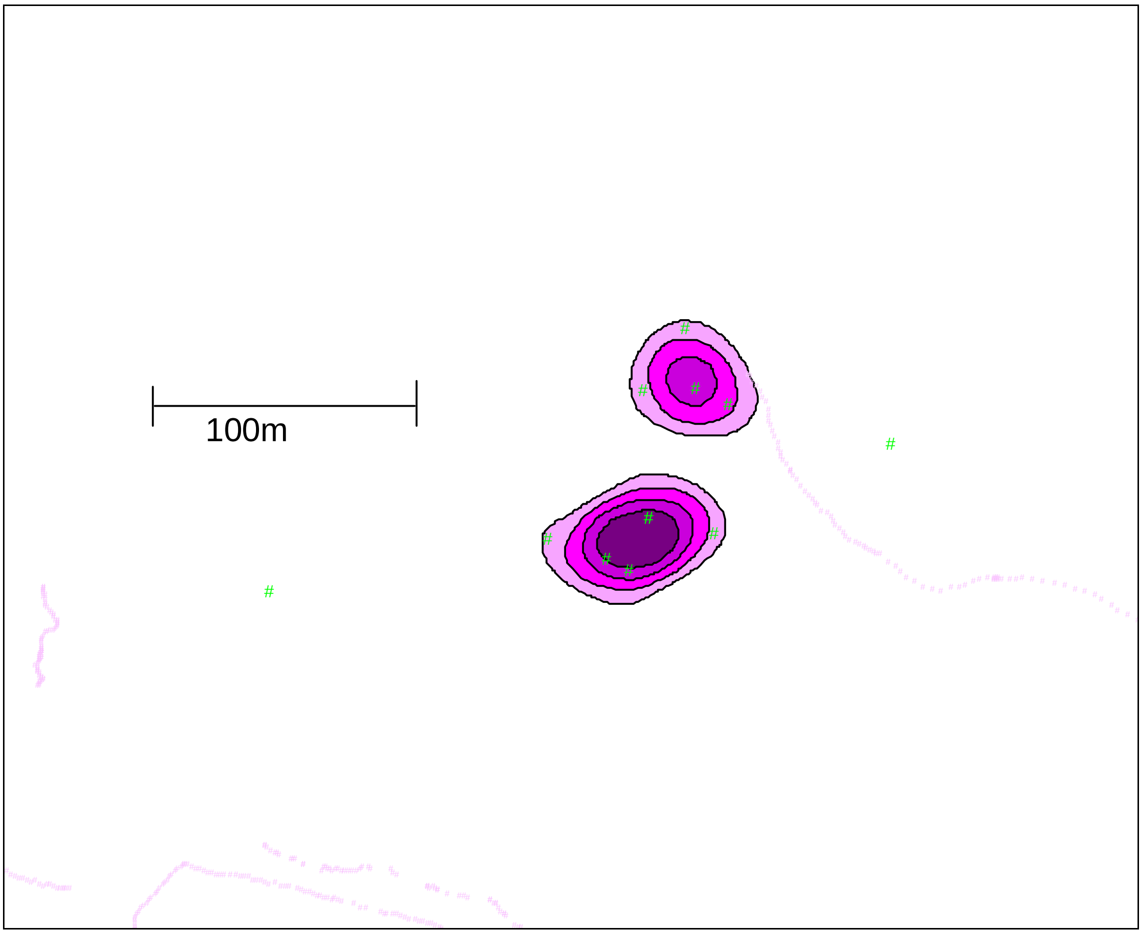


Figure 9. The denning range of Possum 5700 as an example of a bimodal denning range. Den locations are marked by green dots. Contours represent den site density, from high (central most 20% of den sites, darkest shade), through 40%, 60%, to low (80% of den sites, lightest shade). The dotted lines represent access tracks and fence lines present on the study site. Note the dens which lie outside the denning range

The trimodal form of denning range was shown by two possums. The fourth type, that consisted of several groups of dens dispersed across a comparatively large area is illustrated by Possum 5625 in Figure 10. The denning range of Possums 5625 as an example of a dispersed denning range The total of the four denning areas was 1.2 ha although the dens were spread over 8 ha.

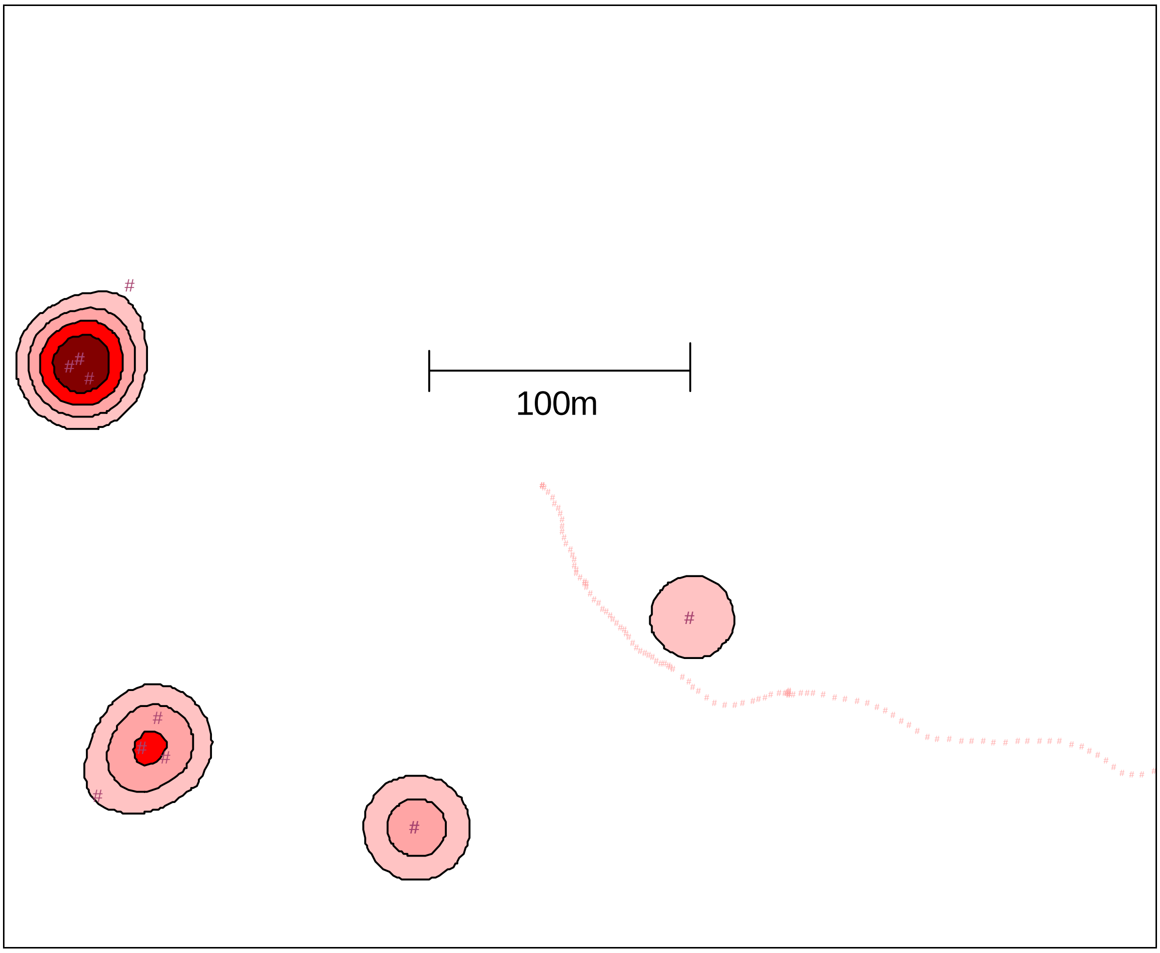


Figure 10. The denning range of Possums 5625 as an example of a dispersed denning range. Den locations are marked by red dots. Contours represent den site density, from high (central most 20% of den sites, darkest shade), through 40%, 60%, to low (80% of den sites, lightest shade). The dotted line represents an access track through part of the study site. The two den sites with a single and double ring around them were used twice and three times respectively

The areas on the study site with high, medium and low den densities each have a similar proportion of possums showing the different types of denning range.

2.3.2.3 Comparison of possum denning range and foraging range

There were 985 trapping records used to calculate trap catch rates. The frequency with which individual traps caught a possum ranged from 0–18. Some trapping records for tuberculous possums were collected before the diagnosis of infection was made. For experimentally infected possums most of the trapping data was collected before the subject was infected.

The possum population did not forage evenly over the study site. (Figure 11). There was one area clearly favoured for foraging and it was characterised by dense scrub with a high proportion of flax, on steep slopes. Areas where possum foraging activity was low were covered by a variety of vegetation types and included pasture on the valley floor. There were three small foci of low use areas. Two were on the top of the western side of Ponga Gully covered by manuka and gorse. The third was on the eastern side of the site, which had a mixture of both open and dense scrub, with small pockets of native bush. The terrain in these areas was of variable slope.

The area of highest foraging activity for all possums as a group was different from the area of highest denning activity (Figure 11). The distance between the two density centres was 200 m and they were separated by a small ridge.

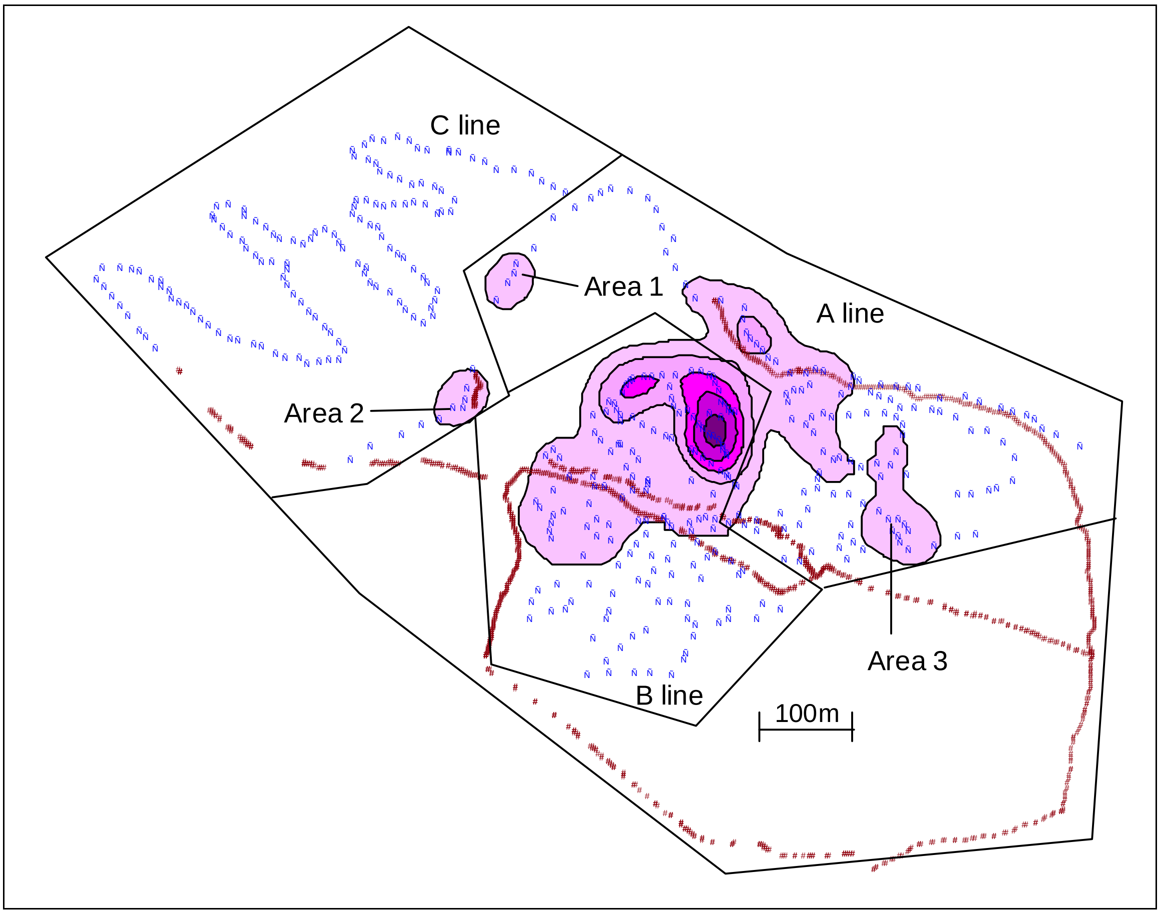


Figure 11. The foraging range of all radio tracked possums on the study site. Crosses represent trap sites, lines define areas covered by the three trap lines. Contours represent density of trapping events, from high (central most 20% of trapping events, darkest shade), through 40%, 60%, to low (80% of trapping events, lightest shade). Areas 1, 2 and 3 represent the small foci of low foraging activity

The denning and foraging areas for individual possums were compared. The centre of the denning area and the centre of the foraging area were less than 100 m apart for all possums, regardless of sex or disease status. There was considerable variation between individual possums in the total area covered by den sites and trap sites. Extreme examples are the patterns shown by Possum 0026, which had a widely dispersed set of den and trap sites, and Possum 0142, which had a tightly clustered pattern (Figure 12).

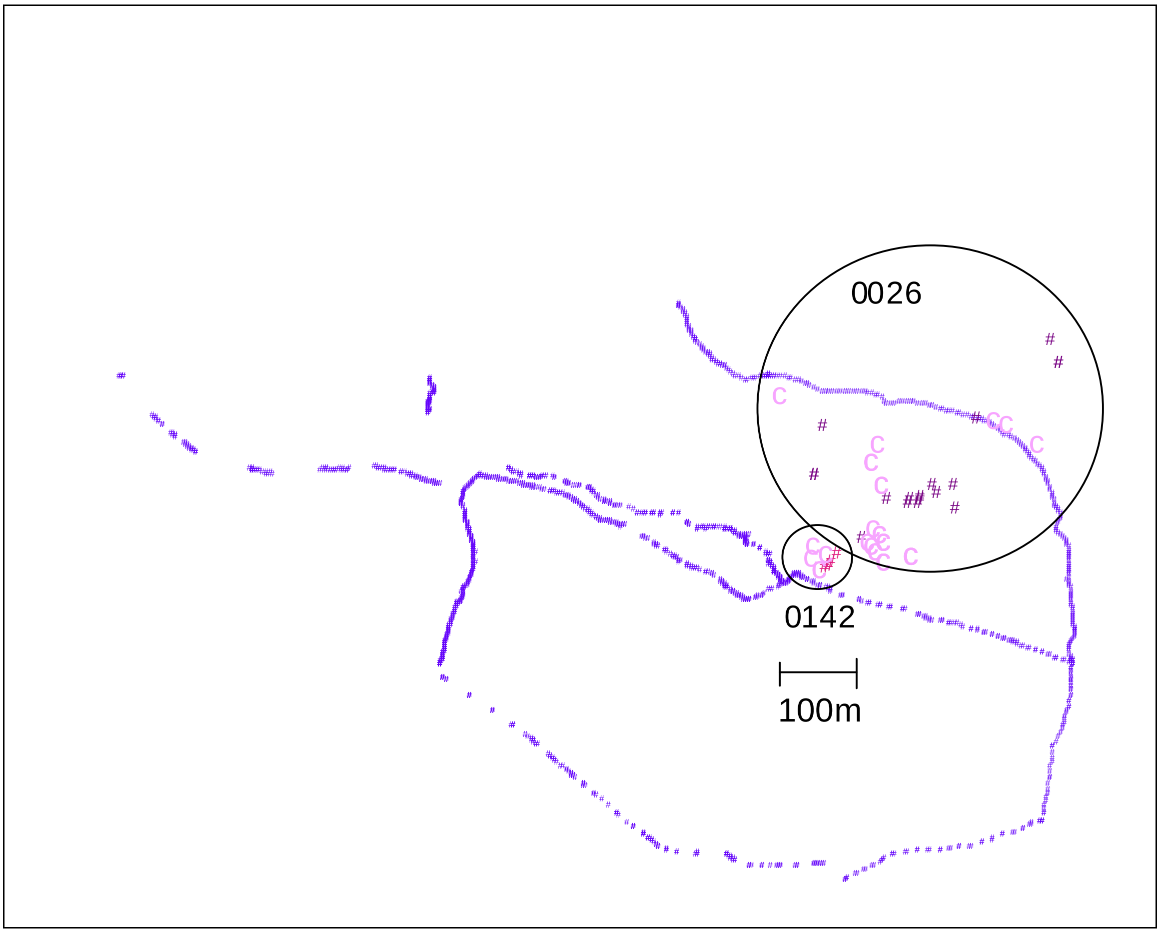


Figure 12. Examples of dispersed (Possum 0026) and clustered (Possum 0146) patterns of denning and foraging by individual possums. Dotted lines = fence lines and reference points, large dots = den sites, flags = trap sites where the possum was caught. Circles encompass the data points for each possum

2.3.3 Activity range and total range of possums

Activity ranges were calculated using a kernel density estimate of combined trapping and denning data. Total ranges were calculated using every data point from trapping and denning data. There was an average of 47 (range 5-89) data points for 28 possums. Two possums were omitted from this analysis because the combined number of data points for each was less than five. The distribution of female activity ranges and female total ranges is significantly skewed.

**The size of activity range and total range was similar for each possum group (
Table 7).**

Table 7. Activity ranges and total ranges (hectares) for three possum groups: naturally infected, experimentally infected and healthy possums

		Activity range			Total range		
Group	n*	Median	Max	Min	Median	Max	Min
Healthy	8	2.2	6.1	0.4	5.9	13.6	1.0
Naturally tuberculous	12	2.5	4.6	0.7	5.3	17.0	1.7
Experimentally infected	8	3.4	6.0	1.6	7.8	10.6	4.8
Overall	28	2.2	6.1	1.6	5.9	17.0	1.0

* number of possums in each group

The activity range of 18 possums was unimodal. Seven activity ranges were bimodal and the remaining three consisted of three distinct areas. A summary and diagram of the activity and total range for each possum is presented in Appendix II, where they are listed in order of possum identification number.

The relative sizes of denning, activity range and total range for each possum are compared in Figure 13.

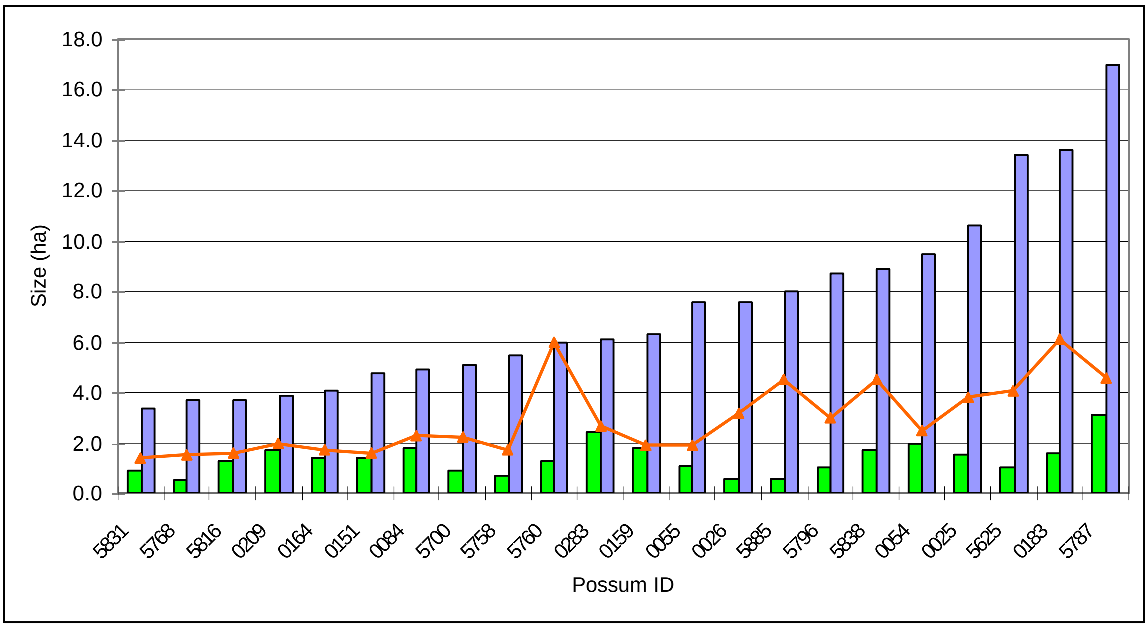


Figure 13. The denning range (green bars), activity range (red line) and total range (blue bars) of 28 possums

2.3.3.1 Correlation between number of observations and range size

**As the number of observations increased, the range size also increased (Figure 14). This correlation was significant for all three types of range (
Table 8).**

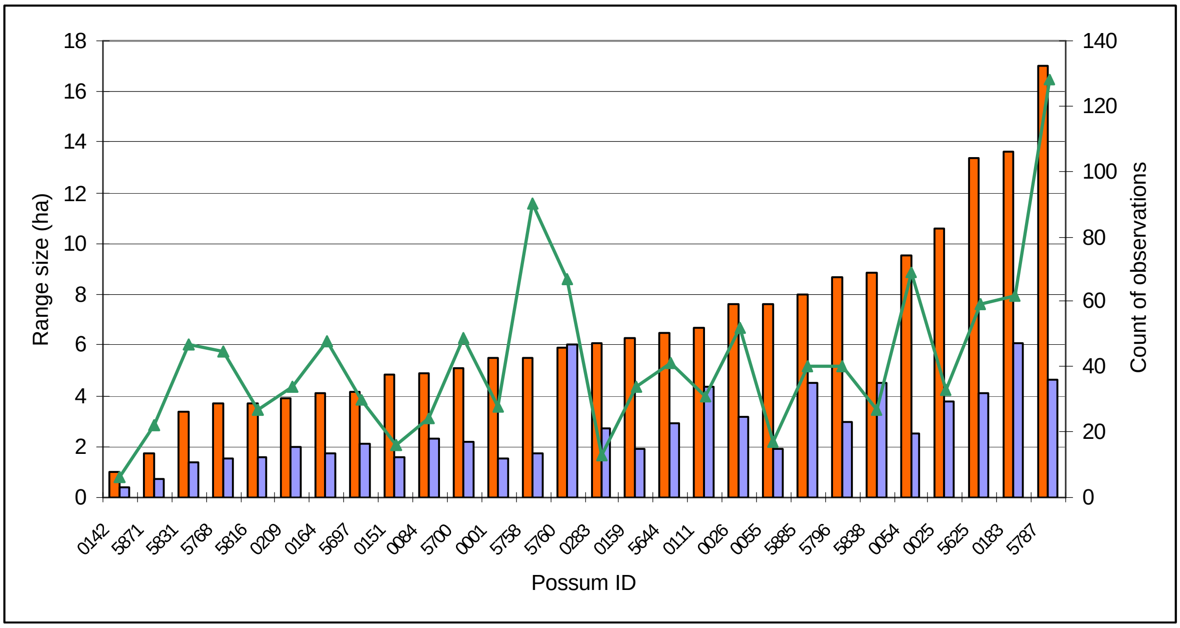


Figure 14. The size of activity range (blue bars), total range (red bars) and number of observations (green line) of 28 possums

Table 8. Correlation between range size and number of observations

Range type	Pearson's correlation statistic	n [1]	P value
Denning range	0.643	22	0.001
Activity range	0.021	28	0.021
Total range	0.613	28	0.001

[1] n = number of possums for which the range type was generated

2.3.4 Long distance forays

Possums infrequently undertook long distance forays from their established activity areas. All radio collared possums were mature. For 28 possums there was sufficient trapping and denning data to examine long distance forays. Half of all radio tracked possums made at least one long distance foray (Table 9). There was no consistency in the direction of these forays. The median distance of forays by tuberculous possums was 290 m (Table 9) which was not significantly different from healthy possums (256m) (Mann-Whitney U=29.5, Z=-1.021, P=0.319). The proportion of each sex making a long distance foray was similar. The median distance travelled by each sex was also similar.

Table 9. The proportion of healthy and tuberculous possums which made at least one long distance foray and the median distance from their activity range

Possum group	n [1]	Percent of group [2]	Median distance (m)
Healthy	8	50	256
Tuberculous	20	50	290
Males	18	56	285
Females	10	40	262

[1] The number of possums in each group

[2] The percentage of each possum group which made at least one long distance foray

Most possums made a single foray and no possums made more than three forays. The frequency of movements of male and female and also tuberculous and healthy possums were similar (Table 10).

Table 10. The frequency of long distance forays made by individual possums

		Number of movements by individual possums		
Possum group	n [1]	Once	Twice	Three times
Healthy	8	2	1	1
Tuberculous	20	7	1	2
Males	18	6	1	3
Females	10	3	1	0

[1] The number of possums in each group

Most forays occurred during February, August, September and October (Figure 15).

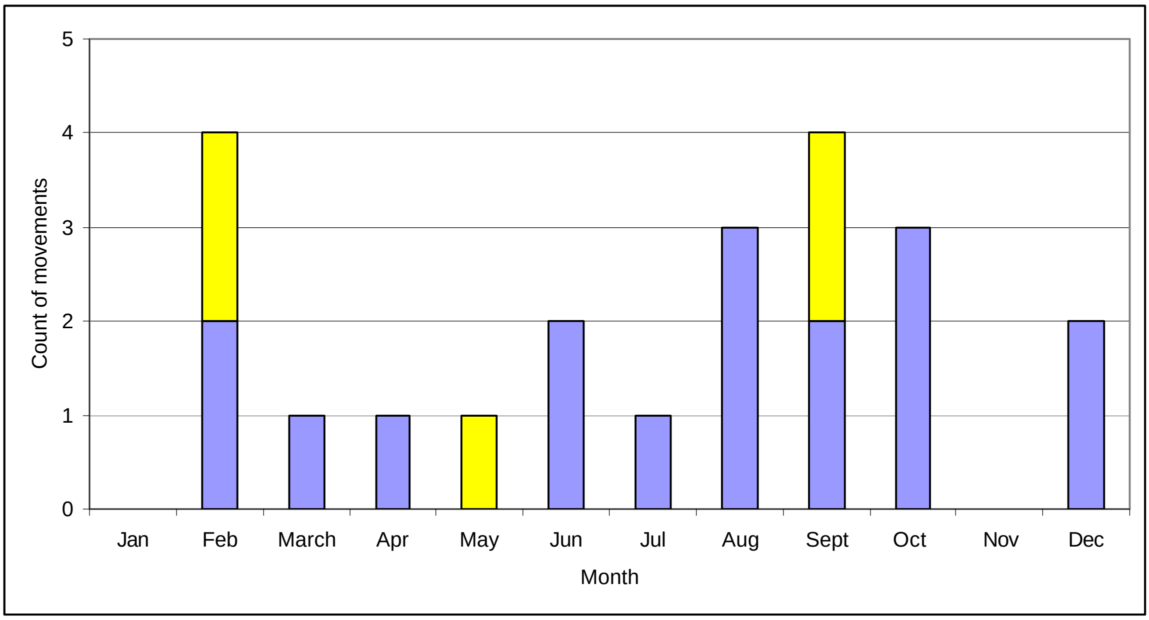


Figure 15. Monthly distribution of 22 long distance forays made by 14 possums. Forays were calculated using trapping data collected over five years and den site data collected weekly over 23 months. Dark segments = males, light segments = females

2.3.4.1 Length of forays

The frequency and distance of long distance forays from the activity range was examined. The length of 22 such forays and a comparison between male and female possums is presented in Figure 16. The distribution of these movements is skewed to the right. The median distance for all forays is 300 m (range 200–660 m). Males moved more frequently than females though the difference is not significant (P=0.164, Z=-1.413, Mann-Whitney U=-24.5).

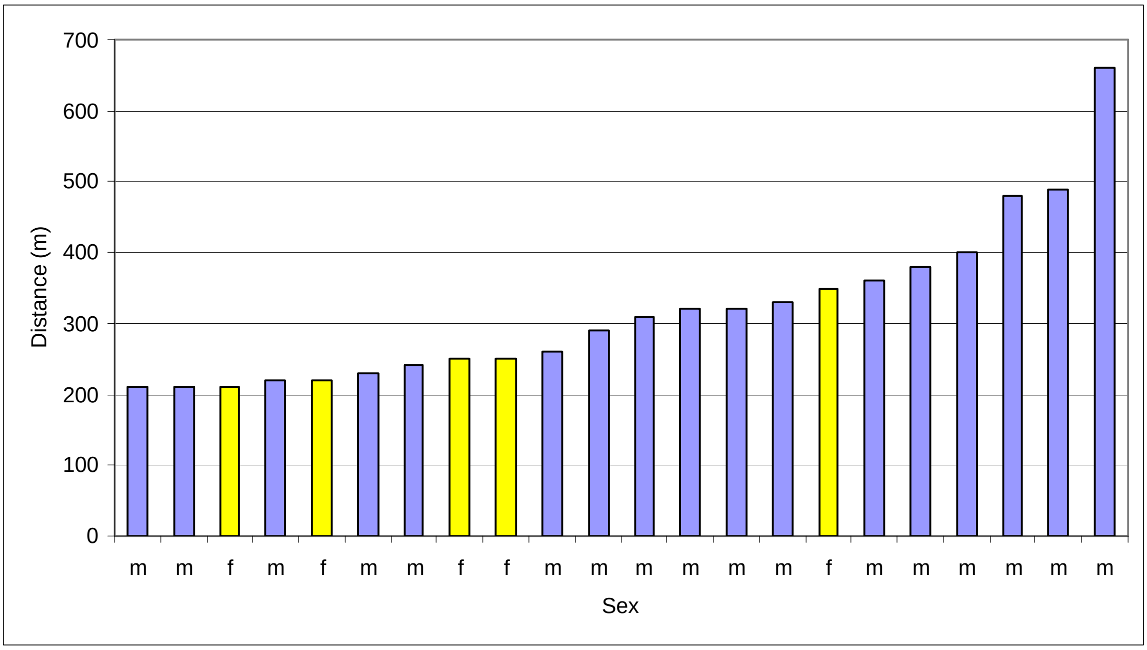


Figure 16. Length of 22 long distance forays made by 14 possums. Long distance forays were calculated using trapping data collected over five years and denning data collected over 23 months. Dark bars = males, light bars = females

A typical example of a long distance foray is shown by Possum 0055 in Figure 17. This possum moved 500 metres to the north west. It was trapped once at this location and subsequently trapped back within its activity range.

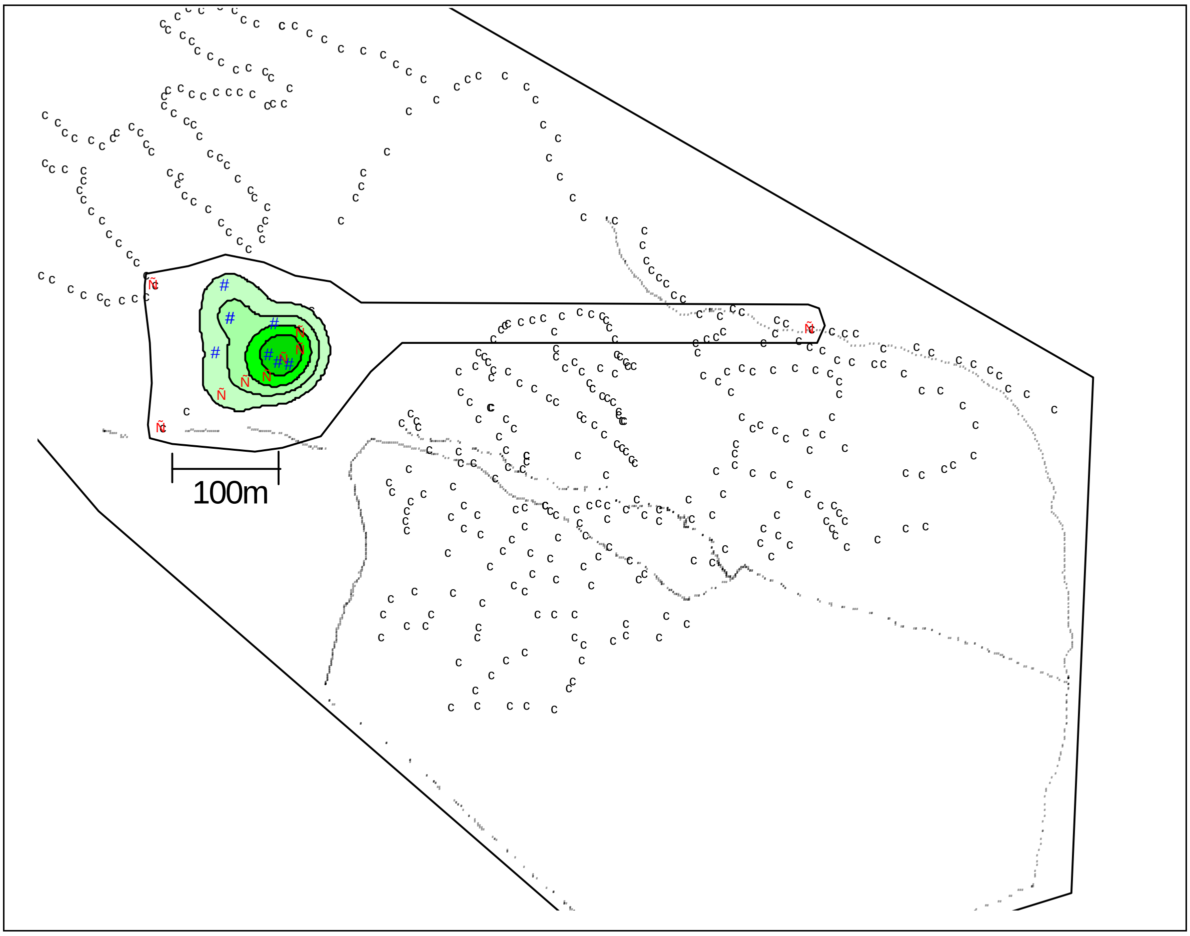


Figure 17. An example of a long distance foray: Traps (red crosses) where Possum 0055 was caught and den sites (blue dots) it was known to use. Contours represent density of combined trap and den locations for Possum 0055, from high (central most 20% of locations, darkest shade), through 40%, 60%, to low (80% of locations, lightest shade). Flags = traps sites, broken lines = fence lines and access tracks

2.3.5 Tuberculosis hot spots

The location of five hotspots on the site identified between March 1998 and January 2000 are shown in Figure 18. Hotspot location was derived from 189 denning events for tuberculous possums. There were two areas of high density, a medium density area and two low density areas. The most prominent hotspot (hotspot 1) was on the steep sides of Ponga Gully. Five of the 22 tuberculous possums favoured this area. The large, central area of medium density (hotspot 3) was used by seven possums, with the denning activity spread over a large area. There were three small hotspots in the north (hotspot 2), east (hotspot 4) and south (hotspot 5) of the study site. The northern focus was used by three possums. Two of these three frequently reused their own tightly clustered group of dens. The tightly clustered group of dens in the east and south were each used by a single possum. The remaining five tuberculous possums used dens within the hotspots, as well as dens where tuberculous possums were seldom found.

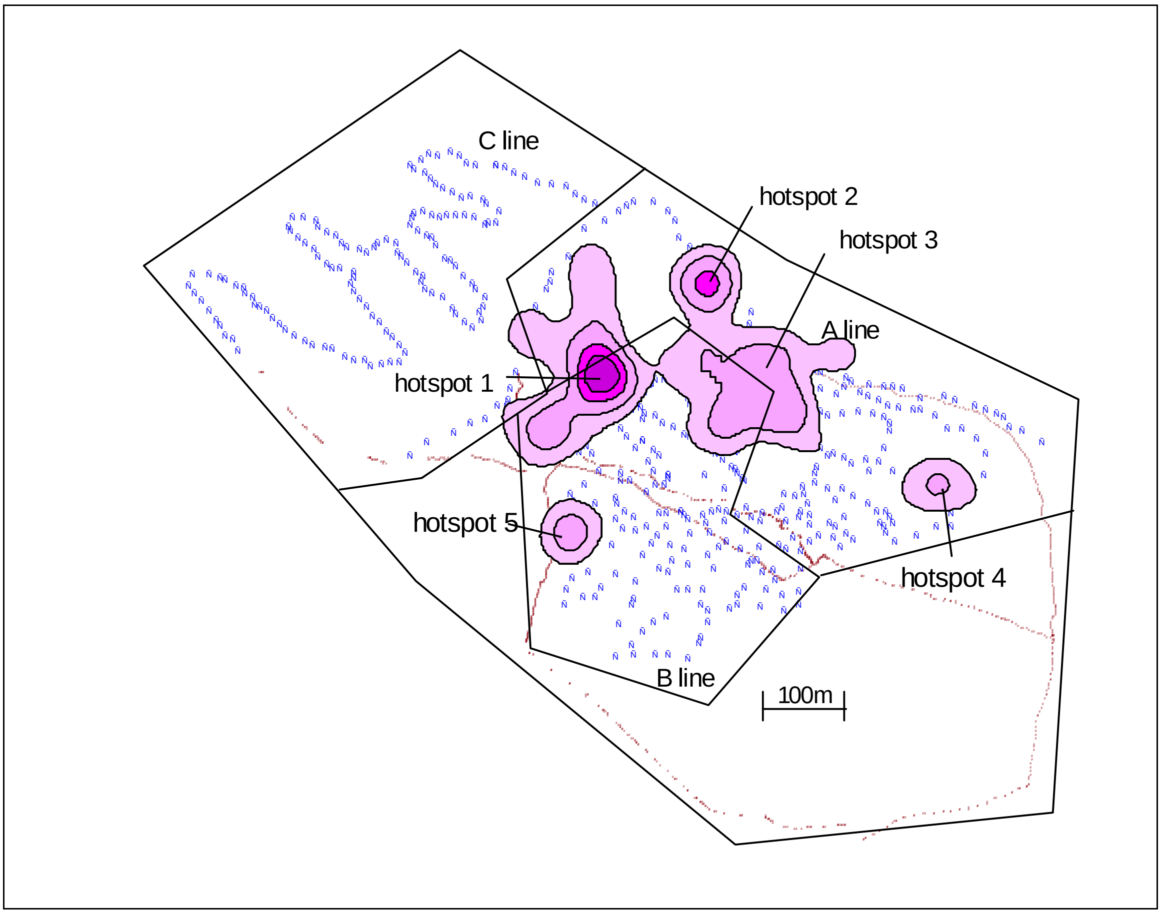


Figure 18. Hotspots identified on the study site between March 1998 and January 2000. Crosses represent trap sites, lines define areas covered by the three trap lines. Contours represent den site density for tuberculous possums, from high (central most 20% of den sites, darkest shade), through 40%, 60%, to low (80% of den sites, lightest shade)

2.3.5.1 Sequential use of the same den by tuberculous and healthy possums

At no time were two or more possums found in a den at the same time. There were 12 cases of two possums using the same den at different times and one case of four possums using the same den at different times. There were five occasions when a healthy possum occupied a den that had previously been used by a tuberculous possum. On four of these five occasions the time interval between diseased and healthy occupations was 1, 2, 23 and 62 weeks. In the fifth case, a tuberculous possum used a den that was then used by three non tuberculous possums at 6, 8 and 18 weeks after the diseased possum had used it. No healthy possums under observation became tuberculous. Dens were used only once by most possums, but in three cases a den was used by the same possum six times (Table 11).

Table 11. Frequency of use of individal dens by possums

Count of times a den was used	1	2	3	4	5	6
Frequency	197	22	8	2	1	3

2.3.6 Den characteristics

Ninety-two percent of dens had a roof that covered ?75% of the den floor, these were defined as fully enclosed. Two percent of 233 den sites had a roof that covered less than 50% of the den floor, these were defined as open den sites. The roof of the remaining 6% of den sites covered between 50% and 75% of the den floor.

The most popular denning material was flax (*Phormium tenax*) and was found in 30% of dens. Gorse (*Ulex europaeus*) was the next most popular at 18%, followed by heaped dead vegetation (13%) and underground sites or recesses in banks (11%). The remaining seven types of den material were only recorded occasionally and are shown Figure 19.

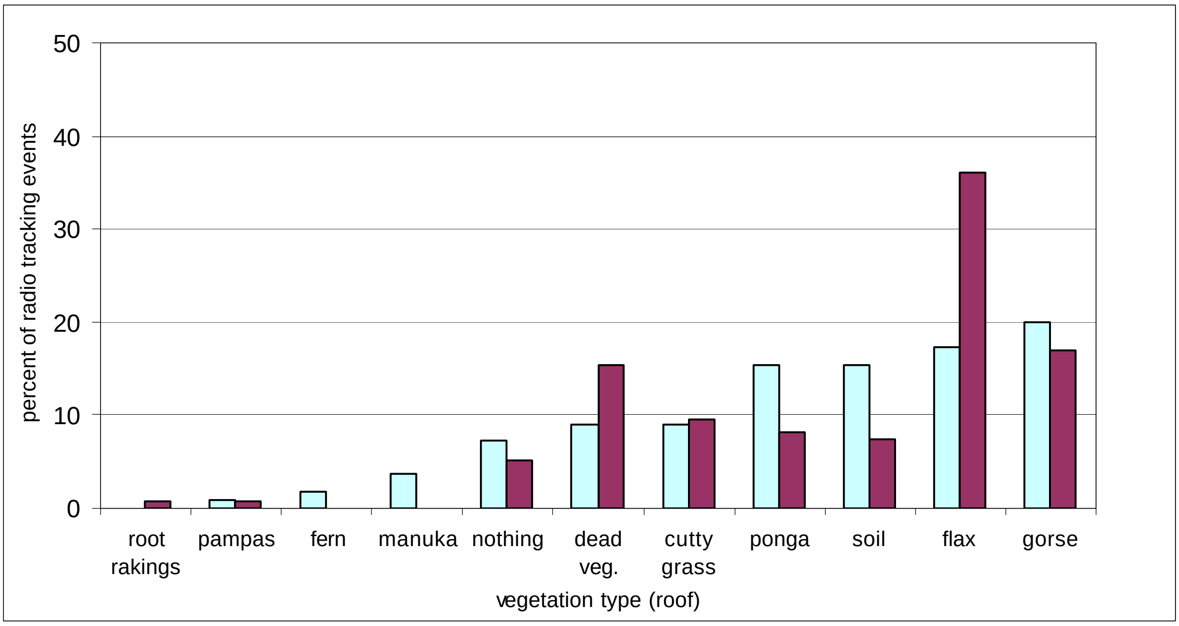


Figure 19. Percentage of denning materials used by tuberculous possums (dark bars) and healthy possums (light bars), the category labelled 'nothing' describes den sites where less than 50% of the sides and roof were enclosed

The direction faced by the slope on which each den was found was recorded by compass bearing, then categorised as having either a predominantly northern or southern aspect (Table 12). Most dens had a northerly aspect. The percentage of north and south facing dens used by naturally infected, experimentally infected and healthy possums were compared. There was no significant preference for the aspect shown by the three classes of possums studied.

Table 12. Characteristics of dens used by possums: aspect of dens used by healthy, naturally tuberculous and experimentally infected possums

Group	North facing (%)	South facing (%)
Healthy possums	57	43
Naturally tuberculous possums	61	39
Experimentally infected possums	69	31

Of the dens identified, 99.5% were at ground level. The floor in most dens (65%) was dry with a few mildly damp (14%) or very damp (15%) (Table 13). Very few dens were classified as wet or saturated.

Table 13. Characteristics of dens used by possums: floor condition of dens used by healthy, naturally tuberculous and experimentally infected possums

Group	Dry (%)	Slightly damp (%)	Very damp (%)	Wet (%)	Saturated (%)
Healthy possums	61	16	16	7	0
Naturally tuberculous	67	11	16	4	2
Experimentally infected	78	22	10	2	0

2. 4. DISCUSSION

Spatial aspects of tuberculous possum behaviour

This study showed that the majority of possums dying from tuberculosis remained within their established activity ranges, in scrub. The carcasses of seventeen tuberculous possums were recovered. Twelve were recovered from scrub and three were found on grass areas within sparse scrub. The carcasses of only two possums were found on pasture, in both cases the possum had moved downhill, and was a short distance (less than that defined by a long distance foray, see section 2.5.6) outside its established activity range. Three possums made long distance forays during the terminal stages of disease but all remained and died in scrub. It had been believed, based on anecdotal accounts, that tuberculous possums migrated from their established activity ranges to areas with easily accessible food and water, pasture in particular. The data from this study show this speculation was untrue.

Tuberculous possums showed a tendency to move to lower elevation as the disease reached its terminal stages. Ten of 17 tuberculous possum carcasses were recovered from the lowest recorded elevation in their range. In three of these cases the carcass was found in a water course and the possums had apparently drowned.

The ranging behaviour described in this study is supported by the results of Paterson *et al.* (1995) who found most tuberculous possums remained within, or close to, their denning range until death. Paterson also found that a small proportion of tuberculous possums made extended forays shortly before death.

Increasing physical debilitation was evident as possums progressed through the terminal stages of disease. Debility was characterised by lethargy, weakness and poor coordination. For possums whose activity range was on steep terrain, their gradual decent to lower elevations during the terminal stages of disease was probably due to physical debilitation. It may also have lead to the cases of drowning.

It is likely that diseased possums descending from high in the scrub to pasture, as a result of debilitation, were responsible for anecdotal reports of diseased possums moving to pasture to feed. The anecdotal observations could have been biased, as possums dying on pasture would be easily observed, unlike those in scrub. The anecdotal reports were also made during a time when the prevalence of tuberculosis was much higher on the study site.

The proportion of terminally ill possums which die on pasture represent a small but serious risk of infection to cattle and deer. During the terminal stages of disease possums are not only debilitated, but are also at the peak of infectiousness (Jackson *et al.*, 1995). The declining physical state results in behavioural changes, such as reduced avoidance reaction to livestock, foraging during daylight and a reduction in activity area (Paterson, 1993). The first two of these changes greatly increase the chances of a normally nocturnal animal encountering and failing to avoid inquisitive cattle or deer (Paterson and Morris, 1995, Sauter and Morris, 1995). In comparison, healthy possums actively avoid livestock. Cattle and deer, attracted by the diseased possum's abnormal behaviour, show a high degree of inquisitiveness and will lick, sniff and even grasp the possum with their mouth. This close investigative behaviour by livestock, combined with the loud hissing and screeching which is behaviour typical of a threatened possum, provide excellent conditions for aerosol transmission of tuberculosis.

Transmission of disease between possums is most likely to occur when the tuberculous possum is still capable of engaging in affiliative or agonistic social interaction, which is prior to the terminal stages of disease (Jackson *et al.*, 1995). The frequency of interactions between a terminally ill possum and other healthy possums is likely to be low as a result of declining physical condition and reduced activity levels.

Tuberculous possums that die in dense scrub represent a lower but variable risk of infection to cattle. This risk is low where cattle are not permitted to forage through scrub, but higher where stock management or poor fencing allows cattle to explore scrub areas. Paterson *et al.* (1995) reports that cattle will investigate bush areas not thought accessible, or attractive to them, increasing the likelihood of encountering a diseased possum. Possums dying in scrub also represent a source of infection to wild animals, particularly deer, or scavenging ferrets and pigs (Wakelin and Churchman, 1991). Tuberculous possums that die in dense scrub represent a low risk of infection to domestic deer. This is because deer are typically fenced such that they cannot forage through scrub areas.

Temporal aspects of tuberculous possum behaviour

Survival of naturally tuberculous possums ranged from one to 84 weeks (median 11 weeks). Survival of experimentally infected possums ranged from nine to 21 weeks (median 12 weeks). Variability in the survival of naturally diseased possums is to some degree artificial as it was influenced to a small degree by radio collars being applied at different stages of disease. Examination of the population for clinically ill tuberculous possums was conducted bimonthly. The period between examinations was large, relative to the survival duration of clinically ill possums, so tuberculous possums could not consistently identified early in the course of the disease. The duration of survival of naturally infected possums in the current study was considerably shorter than that reported by Jackson (1995). He reported the survival duration, following the detection of palpable lesions, to be about six months for 50 percent of animals, with a maximum of 31 months.

Experimental infection of possums resulted in a more rapid disease process than that following natural infection. The survival time after infection in experimentally infected possums was one week longer than the survival time, after clinical diagnosis, for naturally infected possums. In naturally infected possums a preclinical period estimated from studies of captive possums to be eight to ten weeks (Corner, pers. com., 2001) must be allowed for, when comparing relative survival times.

There was one extremely unusual case in which a possum apparently self-cured infection with tuberculosis. Eight months before the beginning of the study Possum 5787 was diagnosed with tuberculosis. Infection was confirmed on two separate occasions by the isolation of *M. bovis*, first from aspirated pus from a lesion, and then 2 months later from a swab taken from an open sinus. This possum survived for the duration of the study and was euthanased during the depopulation. The *post mortem* examination, subsequent histopathology, bacteriology and culture of tissues, all failed to detect any evidence of tuberculosis. Lugton (1997) suggests recovery from infection with *M. bovis* is possible and presented six cases which he believed demonstrated this process. However, in none of these cases was a possum with tuberculosis confirmed by culture, re-examined and found to be culture negative.

The survival period of tuberculous possums is a crude estimate of the period where they may transmit disease to other animals. Transmission to healthy possums probably occurs most frequently during the early to late stages of infection, but before the terminal stages. During this period the infected possum is excreting *M. bovis* but still behaving normally, for example involved in social interactions while seeking a den or during mating. In contrast, the time at which a diseased possum is most infectious to cattle or deer is during the terminal stages, when debilitation limits the ability of a diseased possum to avoid investigation by livestock and levels of excretion are at their peak.

Possum denning, activity and total ranges

The possums in the study were found to den and feed on different parts of the study site. Possums, as a group, most frequently denned in dense scrub on the steep sides of Ponga Gully. This area represents about one quarter of the study site. The area most frequently used for foraging, as revealed by trapping, was 200 m to the east, also in dense scrub. The majority of foraging activity also occurred on about one quarter of the study site. There was a small amount of overlap between areas of low denning activity and low foraging activity.

It is unclear why possums chose these particular areas in which to den and forage as there were few apparent physical differences between the two. There seemed to be a similar number of potential den sites in both areas. Vegetation of the two areas was similar in composition, degree of cover and density. The denning area was extremely steep, compared with the moderately steep foraging area.

The size of denning range was similar across all possum groups. The denning range of an individual possum covered approximately one quarter of its total range, and was located within part of the activity range, but seldom located on its edge, as previously found by Ward (1984) and Paterson *et al.* (1995). There is much data published on the denning habits and home range estimation of possums (see the review by Cowan and Clout (2000)), but little data published on denning ranges. Detailed information of den materials was presented by (Cowan, 1989) but no estimates of denning range were given.

There was much variation in the denning habits of individual possums. The dens used by an individual possum were generally grouped in one to three foci. The size of denning range was similar across these groups. Most possums were found on occasion denning well outside their established denning range. The pattern of den clusters with occasional outliers for individual possums was similar to that observed by Paterson *et al.* (1995). In areas where den density was high the average size of denning ranges was twenty percent smaller than in areas of low den density, indicating that the size of denning range is influenced to a small degree by possum density.

The denning range of individual possums frequently overlapped, both between and within sexes, as did that of healthy and tuberculous possums. In one instance an area of one quarter of a hectare was used simultaneously by four possums in the study, one healthy and three diseased, and quite possibly other possums. The overlap of multiple possum ranges is likely to increase the frequency of interactions between the resident possums. Where a proportion of these ranges are occupied by tuberculous possums, there is a high chance of disease transmission to healthy possums. The demonstration of multiple overlap of denning ranges supports the hypothesis of Pfeiffer (1994) that the transmission rate of tuberculosis between possums is probably highest in areas of favoured denning.

There was considerable range in the popularity of individual den sites. Some dens were regularly reused, up to six times. Some dens were used by as many as four different possums, at different times. Simultaneous den sharing was not observed during the study. Healthy possums were found in dens previously used by tuberculous possums and in two instances the interval between use was less than 28 days. Jackson *et al.* (1995) reported that, under ideal conditions, *M. bovis* could survive for this length of time within a den. When tuberculous and healthy possums frequently re-use the same dens the possibility of healthy possums becoming infected, due to den contamination, is increased. There could also be an increased risk of transmission through aggressive interactions undoubtedly occurring as a result of competition for these favoured dens. Simultaneous den sharing was not observed during this study and has only rarely been observed at the Castlepoint study site in the past (Pfeiffer, 1994). However, it is more common in other areas of New Zealand (Caley *et al.*, 1998). Simultaneous den sharing between diseased and healthy possums would be an excellent environment for transmission of tuberculosis, because of the confined air space and still air which would facilitate the maintenance of infectious aerosols. The proportion of dens used by two or more possums found in the current study was very similar to that found when possum density was much higher in an earlier study at Castlepoint (Paterson, 1993).

The total range and activity range found in this study were substantially larger than those previously reported at this site. The median total range of 5.9 ha and median activity range of 2.2 ha were both greater than the activity range estimate of 1.4 ha for males and 0.9 ha for females by Paterson (1993). The difference in results are due in part to differences in data collection and analytical methodologies. Paterson used radio triangulation to map the nightly movements of possums at fifteen minute intervals. By comparison, the curreent study identified den sites at weekly intervals and collected trapping data at bimonthly intervals. Substantially more data (average 128 points/possum) was generated than in the current study (average 47 points/possum). He found that the range size for some animals stabilised after 100 locations while for others it continued to increase for the duration of tracking (12 months, 458 locations). Range calculation was by the convex polygon, or alternatively harmonic mean method. It is likely that collection of data over long periods, as in the current study, provides a more accurate estimate of possum ranging behaviour than short, intensive observations. Total range values for the current study (which are very similar to home ranges) lay at the high end of home range estimates from other studies conducted in New Zealand and Australia (see the review by Cowan and Clout, 2000).

Long distance forays

Half of all possums made at least one long distance foray during the study. Forays were identified by a distinct movement to a single location, for a brief period and then a return to their activity range. These were made by tuberculous and healthy possums. Males made forays more frequently than females, which supports the findings in a study by Green and Coleman (1984). The median distance was between 250 and 300 metres and the maximum was 660 metres. Travelled distances were longer than those found by Ward (1984), although very much shorter than those recorded for dispersing juvenile possums (Brockie *et al.*, 1987). There was no trend in the direction of long distance forays. The majority of the long distance forays occurred in February, August, September and October. Only three of seventeen tuberculous possums made a long distance foray shortly before death, indicating such behaviour is not induced by the terminal stages of disease.

Most long distance forays were probably associated with breeding behaviour. Forays coincided frequently with the minor breeding season, from August to October, and occasionally with the main breeding season, from March to May. This is consistent with the results of Paterson *et al.* (1995), in which possum ranges were largest during the autumn breeding season. The habitat on the study site is a mixture of scrub and pasture and there were no apparent foci of fruit or nut species, which were claimed to induce movement of possums by up to 1600 m (Jolly, 1976). Faecal evidence indicated that possums regularly visited pine trees to feed on catkins, though these appeared to be nightly visits rather than a relocation over longer periods. All possums were mature, therefore these movements are distinct from the permanent long distance movements characteristic of juveniles dispersing in search of a new home range (Cowan *et al.*, 1997).

Long distance forays are likely to have important implications for the spread of tuberculosis. Travelling possums will pass through the home range of established possums, and agonistic interactions are more likely during this first encounter than with regularly encountered possums in which the social standing of each individual has already been established. If the resident or travelling possum is tuberculous, disease transmission during these encounters has the potential to spread the disease to previously uninfected areas.

There are conflicting views on whether long distance forays should be included in possum ranges. This debate raises the question of whether a range is most accurately represented by all locations for a possum, or alternatively, the area in which the animal spends most of its time. Ward (1984) argues strongly for their inclusion in range estimates, on the grounds that movements to seasonal food sources or during the mating season are important components of possum behaviour. However, the addition of these infrequent, outlying points can have a disproportionate effect on the estimated size of a range, compared with the majority of more centralised points. In the current study, the inclusion of an activity range and total range presents both sides of this argument and allows the reader to come to their own conclusion.

Hotspots

Most den sites used by tuberculous possums were clustered on one quarter of the study site, with a five hectare area of particularly high density. The major hot spot was centred on the steepest area of the study site. Four minor hotspots were also identified on moderately steep to very steep terrain. Vegetation on all hotspots consisted of dense gorse and manuka ranging in height from one to three metres and interspersed with clumps of flax. All hotspots contained a high number of fully enclosed dens. The current study is in agreement with two characteristics of the hot spot predictor developed by McKenzie (1999), the number of fully enclosed dens and the height of covering vegetation, but at odds with the requirement for gently sloping or flat land.

Den sites used by experimentally infected possums were distributed more evenly across the site than those of naturally infected, or healthy possums. It was the goal of the trials to distribute the experimentally infected possums evenly across the study site. Therefore the characteristics of den sites used by this group do not contribute to our understanding of hotspots.

The most popular explanation for the location of hotspots is the association with high numbers of enclosed dens in favourable denning areas. However, this explanation appears incomplete as many other areas on the study site appeared virtually identical in all physical aspects, but supported low or negligible den density for tuberculous possums. One possible explanation is that, in addition to the factors currently believed important for hotspot formation, hotspots are also influenced by the pathways formed and used by wildlife, including migrating possums. Interactions between resident and immigrant possums along these pathways would more frequent than found elsewhere. Where possums are infected with tuberculosis, these spatially concentrated interactions may lead to high rates of disease transmission, and hence a hotspot. A study involving the behaviour of feral urban cats found their home ranges were characterised by a number of points of interest connected by frequently used pathways, with the remaining areas rarely used (Leyhausen, 1965).

In the current study, tuberculous possums denned in some areas also used by tuberculous possums eight years previously. Of the four minor hotspots identified, three were also noted by Pfeiffer (1994). It is not possible to determine whether tuberculous possums had been present in these minor hotspots continuously for the entire period between studies. However it is quite possible that these are permanent hotspot locations, as have been identified in other studies

(Caley *et al.*, 1999). The major hotspot identified in the current study was not mentioned by Pfeiffer. Failure to detect tuberculous possums in this area may have been due to Pfeiffer using fewer trap sites, which unlike the current study, did not enclose the primary hotspot. It appears from this comparison that some hotspots are present for long periods, while others are temporary. To efficiently target tuberculous possums with control operations it is important to periodically re-evaluate hotspot location, to take into account the possible changes in the presence and location of hotspots over time.

This study has provided an alternative explanation for the existence of temporary hotspots. Temporary hotspots were defined as a site where a single diseased possum was found during a cross sectional survey (McKenzie and Morris, 1995). This definition assumes that a temporary hotspot is the permanent residence of a tuberculous possum, but where its activity range does not contain the necessary characteristics to support transmission of disease, therefore the disease is not maintained within that area. The alternative explanation is that a tuberculous possum was trapped while making a long distance foray, during which it did not transmit disease to other encountered possums. The accurate definition of a temporary hotspot is complicated by some characteristics of a permanent hotspot. A permanent hotspot usually functions as a source of moderate to high levels of infection, but may also exist at very low levels of infection, similar to a temporary hotspot, for a variable time period (Coleman *et al.*, 1999).

Hotspots, as areas with high rates of tuberculosis transmission between possums, are most accurately predicted using den site data. Hotspots are frequently defined by trapping data due to the comparative ease with which it can be obtained. The centre of a hotspot was found to vary in location by up to 200 metres depending on whether it was defined using den site data, or trapping data. Transmission of disease is probably highest during agonistic interactions (Pfeiffer, 1994)) and it is believed these are most frequent about favoured denning areas, as a result of competition for den sites, or oestrous females during the breeding season. They may also be due to the establishment and maintenance of social hierarchies. Unfortunately it is substantially more difficult to gather den site data than to collect trapping data. The use of trapping data is a reasonable compromise as long as it is seen as approximating the real hotspot.

Physical qualities of the dens

Possums most frequently used dens in flax, gorse or heaps of dead vegetation, similar to that reported in previous studies at the site (Pfeiffer, (1994), Jackson, (1995), Paterson *et al.*, (1995)). Almost all dens were at ground level and the majority had dry floors, even during winter and after several days of wet weather. Occasionally dens were damp, but very seldom wet. No possum was observed with wet fur as a result of water coming into a den. Most dens had a north facing aspect and a similar proportion of diseased and healthy possums used north facing dens. Good quality dens were still used by tuberculous possums during the terminal stages of disease.

The possum population showed a preference for north facing dens. This preference may be due to the extra warmth and dryness associated with a northerly aspect. In contrast, Pfeiffer (1994) found most possums denned on slopes which faced in an arc between south and north west. This disparity may be due to the methodology by which aspect was determined. The current study recorded the aspect of the half hectare surrounding the den, in this way the highly changeable small scale topography was recorded as close to the den as possible. Aspect over a greater area was recorded by Pfeiffer, which may have caused his results to reflect the general aspect of the site, which was south to north-west.

Limitations of the study

The principal limiting factor in the description of possum ranges was the limited number of subjects and observations on each subject. The effect of this was reduced somewhat by combining trapping records with den site data to generate an activity range. We showed that the greater the number of observations used (maximum 128), and the longer the period of observation, the larger was the range. Even so, the values we have reported for both denning and activity ranges are probably an under estimate of the true values, due to the limited volume of data available for individual possums. (Paterson, 1993) found that, where locations were taken every fifteen minutes over several nights, the range size for some animals stabilised after 100 locations had been determined, while for others the range size continued to increase for the duration of tracking (maximum 458 locations). Although the method of data collection is somewhat different, the impact of increasing observations on expansion of range size is again clearly evident.

Estimates of ranging areas are influenced by the method of data collection, as we have shown and as elaborated by Ward (1984). Disparities occur because there may be a considerable difference between where a possum dens and where it forages (Efford *et al.*, 1994). By investigating the different data types individually and in combination these disparities were demonstrated and an overall view of the various aspects of possum ranging behaviour was shown.

The method of calculation can strongly influence the estimate of range size (Paterson *et al.*, 1995). In this study, point data representing den sites and trap locations was converted into an area, which represents the range, by using kernel density estimates. This method of range calculation has two significant limitations. The first is variation in the estimate due to the number of data points (McCallum, 2000). This effect was minimised by omitting from the analyses possums with less than five records and by manipulation of contour sensitivity, using the H value when calculating a kernel density estimate. The second problem was the exclusion of a small number of outlying points from the estimate of area. These outlying points are extremely important in accurately reflecting all movements of a possum (Ward, 1984). These outlying points were excluded from our calculation of activity range but included in the total range, allowing an illustration of possum ranging behaviour with and without long distance forays.

2.5. CONCLUSION

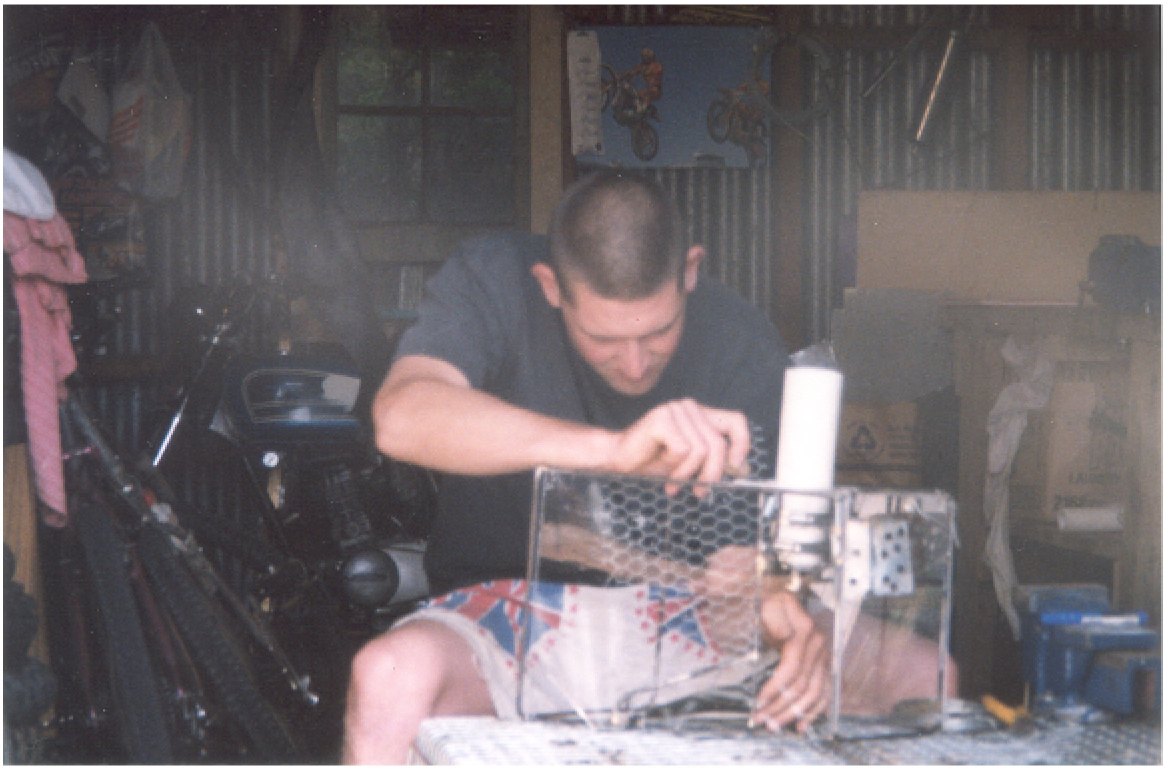
This study used radio telemetry and live-trapping data to observe tuberculous possums and identify aspects of their behaviour that may increase the risk of disease transmission to livestock and other possums. The total range, activity range and den range of tuberculous and healthy possums was similar in size. The behaviour of tuberculous possums was indistinguishable from that of healthy possums for much of the disease process. However, behaviour of tuberculous possums became increasingly debilitated during the terminal stages of disease, which lasted 1 to 3 weeks. Debilitation makes it difficult for a tuberculous possum to avoid investigation by cattle and deer, increasing the risk of disease transmission. In addition, it often causes possums to die at low points of elevation within their normal ranges.

Most tuberculous possums died within or close to their established activity range in dense scrub. However, a small proportion died on pasture. Although the number of tuberculous possums dying on pasture was small, they constitute an important source of infection to livestock. Those dying in scrub are a potential source of infection to wild animals such as deer, ferrets and pigs. A small proportion of diseased possums made long distance forays of 200 to 300 metres in the last few weeks of life, but still died in scrub. There was no temporal pattern in possum deaths.

Tuberculous possums used den sites that were clustered into one major and four minor hotspots. A previous study on the same site had found tuberculous possums in three of these minor hotspots. The use of den site data, or alternatively trapping data, to define the location of a hotspot caused variation of the location by 200 m. Den site data provides a more accurate definition of hotspot location than trapping data due to disease transmission between possums being most frequent in denning areas.

Chapter 3

DESIGN AND TESTING OF A SELF-SETTING AEROSOL VACCINATOR



The spacious, clean, fully equipped work space for vaccinator construction. Leaving the garage door open provided enough light to work by, while Cyril the motorbike crouches in the background.

3.1. INTRODUCTION

Tuberculosis (*Mycobacterium bovis*) was probably introduced to New Zealand with imported cattle during the 19th century. Today the disease is present at low levels in domestic cattle and deer herds and represents a potential threat to the country's large export industry. The test and slaughter method plus restrictions on the movement of animals from diseased herds are used to control the disease in livestock.

The brushtail possum (*Trichosurus vulpecula*) was introduced to New Zealand during the 1850s. This highly adaptable animal quickly spread over much of New Zealand and in 1985 had an estimated population of 70 million (Cowan and Bayliss, 1998). Possums infected with tuberculosis were first discovered in the wild in 1969 (Ekdahl *et al.*, 1970). The disease is now considered endemic in possums over approximately one third of New Zealand (Animal Health Board Annual Report, 2000). The possum is considered a wildlife reservoir of tuberculosis, acting as a source of infection to livestock and wildlife. The control of tuberculosis is based on the large scale aerial spreading of poison baits for possums and the test and slaughter method for infected livestock (Montague, 2000) .

Reduction, or ideally removal, of the wild life reservoir of disease would greatly improve the effectiveness of test and slaughter methods, possibly leading to eradication of tuberculosis from livestock in New Zealand. In Australia, eradication of tuberculosis from wildlife in particular Swamp buffalo (*Bubalus bubalis*), has facilitated the eradication of the disease from domestic livestock (Cousins *et al.*, 1998).

In Great Britain and Ireland, the badger (*Meles meles*) is associated with persistent infection of livestock with tuberculosis, in much the same way as the possum in New Zealand (Cheeseman *et al.*, 1989).

In its national pest management plan for the years 2000–2011 the Animal Health Board proposes expenditure on possum control to average 60 million dollars per annum. The total proposed annual expenditure for the control of tuberculosis in New Zealand is 85.5 million dollars with possum control being the major component. The plan is to use existing control techniques but at higher intensity (Animal Health Board, 2000). Existing control techniques have reduced the prevalence of tuberculosis in livestock, but have failed to control geographical spread of the disease in wildlife.

Bacille Calmette-Guerin (BCG) is a live vaccine which has been used to protect humans against tuberculosis for many years. It has also been shown to protect a range of animals, including possums (Buddle *et al.*, 1997), cattle (Buddle *et al.*, 1995). Vaccination of cattle or possums is a possible alternative to current disease control techniques. However, there are two major complications facing the vaccination of domestic animals. Firstly, immunisation of cattle with BCG results in some animals displaying positive skin test responses to bovine PPD (tuberculin). The tuberculin test is currently used to identify diseased animals. If vaccination of livestock were to be implemented, a new method for detection of tuberculous animals would be required. In addition, the large-scale vaccination of cattle would require approval by national and international regulatory bodies on the grounds of food safety. Secondly, the issue of tuberculosis in wildlife species would not be addressed and current control operations would be required to continue indefinitely.

To avoid the problems inherent in vaccination of livestock, it may be preferable to vaccinate wild possum populations. Research has shown that delivery of vaccine as an aerosol to the respiratory tract and conjunctiva of the possum protects against disease (Corner, pers. com. 2001). Computer modelling, based on the concept of herd immunity, suggests that when the protected proportion of a possum population reaches 50–70% the disease can no longer persist within the population (Barlow, 1997). Vaccination would probably be used in conjunction with existing control techniques.

The vaccination of wild fox (*Vulpes vulpes*) populations against rabies using oral baits has proven an effective method of disease control in several European countries (Aubert, 1996). In addition, it has provided a model on which to base possum vaccination.

There are two key components required for the successful aerosol vaccination of wildlife. One is a simple form of aerosol dispenser and the second is an aerosol can containing the vaccine. The following chapter describes the development of a self-setting aerosol vaccine dispenser (vaccinator) to suit delivery to wild possum populations.

Part one: Pen trials of an aerosol vaccinator

3.2 OBJECTIVE

The objective of this study was to design and refine a self-setting aerosol vaccinator. To achieve this, the study had three interdependent aims.

1. To study the investigative behaviour of possums toward novel objects.
2. To construct a vaccinator. This device had to meet five essential design criteria:

   ?? It must be attractive to possums

   ?? It must be easily used by possums

   ?? It must apply an aerosol spray to the face of the possum. In pen and field trials, the use of aerosol cans containing dye required that the spray be directed at the head and shoulders of the possum to conform with animal ethics requirements.

   ?? The device must be simple

   ?? It must be robust
3. To evaluate the influence of social hierarchy on vaccinator use by captive possums.

3.3. MATERIALS AND METHODS

Pen trials were conducted between June 1998 and February 1999. Observations were conducted weekly for a period of approximately three hours. An observation period began when possums first emerged from their dens and finished at the conclusion of their first active period.

The observation pen was 12 x 15 m and 2 m tall. The floor was a mix of soil and vegetation. There were several raised wooden runways constructed throughout the pen. Possums denned in hessian sacks which hung in two weather proof shelters within the pen. Possums were fed daily with a mixture of fresh fruit, vegetables, bread and cereal-based food pellets and water was available *ad lib*.

A small hut functioned as part of the pen wall and had a large hatch which opened into the pen for weather proof observation. Observation equipment consisted of two small floodlights mounted on the pen roof. These were covered in red cellophane and powered by a 12 volt battery. Light was focused on approximately one quarter of the pen area where the ground was gently sloping and free from obstacles. Novel objects and vaccinator designs were presented in this area. There was also sufficient light provided to view possum activity in ninety percent of the pen. Observations were recorded by video camera and written notes.

An identity collar positioned a reflective number above the head of each possum, enabling accurate identification of individual possums at low light levels. The collar consisted of a plastic zip-tie, 8 mm wide. Attached to the end of the zip-tie mast was a thin plastic plate 45 mm x 45 mm. Attached to both sides of this plate was a number or letter, cut from reflective adhesive. A small fishing sinker (15 g) was also attached to the zip tie. This acted as a counter weight, keeping the reflective tag above the head at all times. Once an identity collar was applied, the ratchet tightening mechanism of the zip tie was wired in place ensuring that the collar could not be tightened if it became caught, for example in the wire netting of the pen.

The social interactions of captive possum colonies were observed during their normal nightly routine and also during the investigation of various novel objects and vaccinator designs. The spectrum of interactions were ranked in a system based on the study by Oldham (1986) presented below.

1. **Avoidance**. Actively moving around the space of another animal to avoid a confrontation. The radius of this space was 1 m.
2. **Appraisal**. A nose to nose, or sniffing appraisal of another animal with no other obvious physical interaction.
3. **Threat**. A defensive position using one or both forepaws, this may be accompanied by aggressive screeching and the occasional light cuff, or swipe which is not reciprocated.
4. **Attack**. Includes a pouncing movement, often from the side or back of another animal. Biting and grabbing, often accompanied by some form of chase as the subordinate animal attempts to escape.
5. **Fight**. A fully reciprocated attack where both combatants bite, scratch and kick, often as they roll around on the ground. Vocalisations vary, but are often grunts and screeches.
6. **Compliant**. Where two or more possums occupy the same space and/or object without any aggression. This is distinct from appraisal as each party has been identified with no ongoing investigation. The attention of individuals is focused on something other than possums.

Four possum colonies were used. There were fourteen possums in three colonies and twelve possums in the fourth. Three colonies were entirely composed of males and one was largely female with a single male. Each colony was observed on average seven times over eight weeks.

Chocolate was used as an attractant during all other observations except during the first three observation periods and presentation of the first vaccinator design, when no attractant was included. Cinnamon powder was used to dust the second vaccinator design presented, but not used after this. Attractant was positioned to be inaccessible to investigating possums and was seldom recovered.

During the first three observation periods no object was presented. Following this, novel objects of gradually increasing complexity were presented. This series began with a heavy plastic cube with sides of 200 mm and progressed to include a bucket, a chair, a gumboot, one metre lengths of pipe, 15 cm or 25 cm diameter, used as tunnels, with both open and closed ends and finally a ball made from wire mesh, containing attractant, hung 90 cm above the floor of the pen.

During the second series of observations thirteen variants of vaccinator design were tested. The degree to which each vaccinator met the design criteria and areas which required modification were determined by observation of possums interacting with the various designs.

Vaccinator designs were constructed from 6mm steel rod and 25 mm x 3 mm steel plate. Additional materials included transparent perspex, wire mesh, 2 mm galvanised iron, electrical double plug boxes, light springs, 2mm aluminium plate, tie wire and assorted fasteners. An oxy-acetylene gas set was used for brazing and welding.

3.4. RESULTS

3.4.1 Observations of possums exposed to novel objects

Observations over a total of 37 hours were used to record the behaviour of captive possums in response to novel objects, before the first vaccinator was presented.

The first three observation periods were used to study possum behaviour in a familiar environment without a novel object. Possums were divided into three broad categories depending on overall behaviour. The first category contained most possums (70%), and was characterised by active investigation of surroundings, particularly food bowls and scent marking areas. Possums in the second category (20%) were less active and would remain stationary for up to 1 hour, at favoured positions on runways. Visits to food bowls were brief with a small amount of exploratory behaviour followed by a return to the favoured sitting area. These possums were alert and observant of the activity and vocalisations of other possums within the pen. There were few possums in the third category (10%). They were active, though showed very little investigatory behaviour. These individuals would spend much of the observation period moving around the perimeter of the pen or pacing up and down a runway. All possums were strongly attracted to large flying insects, cicadas in particular, which were caught and eaten.

?? Category one: possums showing active investigation of surroundings and novel objects.

?? Category two: possums showing some investigation of their surroundings and novel objects, but also spend long periods stationary.

?? Category three: possums active but displaying minimal investigative behaviour of novel or familiar objects, and repeated pacing of runways, or circling of the pen perimeter.

Presentation of a novel object initiated a clear pattern of investigatory behaviour. Possums in the first category would be the first to investigate the object. The possums would approach the object in a largely repeatable order. When most of the first group had completed their inspection and had moved away, possums in the second category would approach. Possums in the third group would rarely investigate novel objects.

A heavy cube was the first object presented. In most cases the investigation lasted less than thirty seconds. Investigation was primarily by smell as the possum circled around the object before moving away. On some occasions an individual would briefly chew a corner of the cube.

An inverted plastic bucket was the second object presented and this resulted in much more intensive attention (maximum 10 minutes) shown by all investigating possums. Possums in category one quickly learned the most effective way to recover the attractant, which was placed under the bucket. A weight placed on top of the bucket ensured that possums could no longer recover the attractant.

The bucket containing the attractant was placed inverted on the seat. This arrangement demonstrated the willingness of possums to climb on novel objects while exploring them.

Investigation was thorough and included smell and taste while seeking the attractant. The chair was frequently tipped over but possums were not deterred by the occasional fall.

Presentation of a gumboot and two pieces of pipe showed that possums will investigate small openings. The more active individuals in particular forcefully pushed their way into the confined spaces. When pursuing the attractant in a gumboot, at times only the back legs were visible, slowly propelling the gumboot and possum across the ground. The 15 cm diameter pipe was not quite large enough for the bigger possums to enter, however they would attempt to squeeze in as far as possible. All possums could easily enter the larger pipe. Approximately 20% of studied possums walked through the pipe and would frequently pause in the middle while sniffing the inside of the pipe. These possums were all in category one. Possums in category two and three were much more apprehensive when exploring small spaces compared with open objects such as the chair.

A wire mesh ball containing attractant was suspended 90 cm above the pen floor. It could be seen and smelled by the possums, but was out of reach. Many possums would sit underneath the ball and make upward grasping movements with both paws. In one case a possum repeatedly climbed a post 1.5 m away and made grasping movements from the post. During the observation period up to five possums would sit or circle beneath the ball. In the last fifteen minutes, one individual jumped straight up and grasped the ball which detached from the string and fell to the ground. During a second observation period using the same object no possums were seen to jump for the ball.

3.4.2 General observations on the approach and investigatory behaviour of possums

In summary, the basic behaviour of individual possums was consistent across the four observed colonies. General patterns observed in response to novel objects are listed below.

1. There was a range of investigative activity between individuals. Some will directly approach and inspect a novel object, while others actively avoid anything new and maintain a distance of at least one metre from the object.
2. During an investigation a possum’s ears were pricked forward, with constant and sometimes loud sniffing as it approached the object.
3. A possum would sniff and inspect the ground along the path of approach as it moved toward a novel object.

4. The scent of other possums on a presented object appeared to reduce wariness of investigating individuals, even when the scent was from a different group of possums.
5. Some possums would approach a novel object several times before apparently becoming apprehensive and moving away.
6. A possum may stretch it’s head and neck forward to smell a novel object, making its body low to the ground. This allowed a rapid withdrawal from the object if required.
7. Possums often made a preliminary inspection by circling the object from a distance of 30–50 cm, before commencing closer investigation.
8. A clear escape route appeared to reduce the level of apprehension in investigating possums. Steep or rough ground and the presence of other obstacles which could not be readily climbed seemed to increase apprehension.
9. Possums appeared to use a combination of senses during investigation, in estimated order of importance they were smell, taste, sound and sight.
10. The degree of apprehension shown by a possum when approaching or investigating a novel object appeared to increase when other possums were within approximately 1.5 m.
11. Possums seemed to check locations where attractant had previously been found when presented with a familiar object. These locations would be checked up to five or six times during total inspection.
12. In each colony of possums, approximately eighty percent would investigate a novel object, however, approximately twenty percent would not approach the area used for presentation of objects. Avoidance behaviour did not change when observations were conducted without artificial light, or when no novel object was presented.

3.4.3 The influence of dominance hierarchy on the investigation of novel objects by possums

Four captive possum colonies were used to observe social interactions over a total of 58 hours. Observations were recorded when there was no novel object present, and when a range of objects including vaccinator designs were presented. Observations were condensed to highlight a number of trends which are listed below.

1. Interactions between two or more possums appeared to begin when their individual space overlapped. The size of this space was approximately one metre and was determined by the maximum distance at which one possum would react to the presence of another.
2. Each possum colony contained one clearly dominant animal. The degree of aggression shown toward subordinate possums seemed to vary between colonies.
3. The hierarchy below the dominant animal (middle order animals) was subject to change. The outcome of most middle order agonistic interactions was dependent on the strength of position of the individuals specific to that encounter. For example, the successful possum would have a tactical advantage, such as attacking the hind quarters or from an elevated position.
4. In two colonies there was one particularly small possum. In both cases it was at the very bottom of the social order and displayed affiliative behaviour such as food sharing and nose to nose sniffing. In the other two colonies no possum was clearly at the bottom of the social order.
5. During most agonistic interactions a low level of aggression was sufficient to determine the dominant animal, for example a threatening posture, hiss, pouncing motion or short chase. During 58 hours of observations only three interactions were clearly classed as fights.
6. The social activity of male colonies and female colonies was different. Up to six males were observed investigating an object simultaneously with much overlap of individual space but only a small amount of low level aggressive behaviour. Females demonstrated less overlap of individual space, more frequent agonistic interactions and a higher level of aggression during these interactions. As a result, a maximum of two or three females would investigate an object simultaneously.
7. The dominant possum was first to inspect anything new in three of the four groups. Subordinate animals were not permitted to approach until the dominant animal had completed a thorough inspection. At the conclusion of investigation the dominant animal would sometimes remain near the object, in acceptance of the presence of investigating subordinate possums. Other times it would move away at which point subordinate possums would approach. In the fourth group one middle order possum would repeatedly be first to investigate anything new, but would quickly be chased off by the approaching dominant animal.
8. Over the course of an observation period all possums attracted to an object would have the opportunity to inspect it. In some cases, groups of up to six possums would investigate

simultaneously. On the other hand, some subordinate animals would wait for several hours to allow a solitary investigation with minimal disturbance.

9. Possums seemed highly aware of other individuals when approaching an object, and subordinate possums appeared more apprehensive when making an approach in close proximity to a dominant animal.
10. Subordinate possums approached a dominant animal toward the side or hind quarters where possible. They appeared to avoid approaching the head of the dominant animal directly.

3.4.4 Pen trials of an aerosol vaccinator

3.4.4.1 Introduction

The initial concept was to design and build a self-setting aerosol delivery device (vaccinator), triggered by a possum. This concept was then converted into five design criteria based on the functions the device was expected to perform (listed in section 2.0). A literature review provided information on the methods used for aerosol vaccination. Aerosol generators described were of two broad types. Those comprising the first type were simple, hand held atomisers (Valois Spray atomisers, Douglas Pharmaceuticals Limited, Auckland, New Zealand) or devices for intranasal instillation (Fort Dodge Pinnacle I.N., Pacificvet Limited, Christchurch, New Zealand). These products were designed for manual operation and used when handling sedated animals. Those comprising the second type were large, complicated and extremely expensive machines. For example the Airborne Infection apparatus (Middlebrook, 1961), Turbair Vaccanair apparatus (Latif *et al.*, 1981) and the ultrasonic nebulizer (Lagranderie *et al.*, 1993). Neither of these two types provided a practical starting point from which to develop a mass producible, self-setting aerosol vaccinator suitable for use in the wild. An aerosol delivery system has been designed for vaccination of pigs (Brown *et al.*, 1997). Parts of this design were relevant to the current project, for example it was propellant driven and designed to deliver aerosol droplets of 5 ?m containing bacteria, to the respiratory tract. Unfortunately the device was hand held, hand operated, mechanically complex and delicate. Finally, it required batteries and restraint of the animal. These factors made the device unsuitable for the self vaccination of wild possums.

The five design criteria were used to construct the initial prototype which was based on a modified cage trap. The developmental process began from there and was directed by observation of captive possums interacting with presented designs during pen studies. All designs incorporated an attractant of chocolate which was inaccessible to possums in most cases. The end point of development during the pen trial phase was replication of the final design of the vaccinator in preparation for the first field trial. The first design was constructed in November 1998 and modifications were tested weekly until February 1999.

3.4.4.2 Type 1 MkI

The initial design was developed in consultation with Rod McDonald and Joanne Short of HortResearch, Ruakura, Hamilton, New Zealand and Leigh Corner, EpiCentre, Massey University, Palmerston North, New Zealand. It consisted of a walk through passage with one way entry and exit doors. Where the aerosol can was activated by the weight of the closing entry door. Characteristics of the initial prototype are presented below.

?? A live animal trap was converted into a rectangular passageway, with one way entry and exit doors.

?? It was a 240 mm square passage, 500 mm long.

?? The weight of the entry door falling shut triggered the aerosol can by way of a wire loop from the door to the can nozzle. A plastic guide pressed on to the top of the aerosol can, kept the wire trigger in contact with the valve of the can.

?? Within the passage way was an enclosed space which housed the trigger and aerosol can. It was accessed through a small door in the roof.

?? The doors and roof were made of sheet metal and the sides and floor were wire mesh.

Aerosol can selection

The primary criterion for can selection was a design currently mass produced and requiring a minimum amount of energy to activate. There are many shapes of can available, but essentially only two types of valve. These are vertically depressed and tilt action valves. The sideways movement of a tilt action valve requires considerably less force to activate than a vertically depressed valve. The long stemmed nozzle used for vertical release of tilt action valves provided more leverage than nozzles designed for the horizontal release of these valves. It was also easier to attach a trigger to the long stemmed nozzle. The aerosol can selected was the ‘Airozone’ brand, 175 grams, using hydrocarbon as propellant and produced by Reckitt and Colman, Auckland, New Zealand.

Observations

The initial prototype was not attractive to possums. The most frequent response (10/14 possums) was to avoid the area completely, or pass in an arc around it. Four possums in category one came within 1.5 m of the device, stopped and sniffed in its direction before abruptly change in direction to avoid it.

Before the second trial, the prototype was thoroughly cleaned with soap and water followed by dusting with cinnamon. Possums were attracted to the traces of cinnamon, sniffing more strongly about these areas. However, the entry door which had to be pushed open acted as a barrier. Possums moved around the device, including sitting on top but made no effort to push against any part. On occasion a possum would move as close to the bait as possible and reach through the mesh in an attempt to rake it closer.

Conclusion

During the first trial the main problem appeared to be the smell of either the vaccinator or the aerosol can. The can was briefly discharged in a test prior to the beginning of the observation period. However, once possums were attracted to the device, it became clear that they would not press against a barrier to gain entry. The whiskers about the nose, eyes and fore-paws of the possum are a highly sensitive method of identifying barriers during nocturnal activity. The force required to push the door open comes from these sensitive areas, which identify the door as a barrier. Observations with novel objects have shown possums will push their way into various things, for example a gumboot or narrow pipe. It was concluded that the important difference was the tapered entry into the boot, compared with the flat surface represented by the vaccinator door.

The first prototype met the first design criteria, being attractive to possums, but failed the second of useability, due to the concept of entry by way of a door.

3.4.4.3 Type 1 MkII

Figure 20. Type 1, Mk II vaccinator

Changes

?? Steel doors were replaced with transparent, slightly lighter perspex copies.

?? The cinnamon was removed.

?? Chocolate was used as an attractant.

Observations

Possums were not encouraged to push against the door by being able see through it. Possums sniff with similar intensity around the whole of the device.

Conclusion

It was clear that possums were not prompted to push against the door by being able to see through it. The focus of attention was directed at the vaccinator, but required further direction toward the door. With the current model the attractant diffused through all parts of the device. It was possible that the solid perspex doors encouraged possums away from the entrance as the scent would have been stronger from the porous mesh sides. This model (**Figure 20**) did not satisfy any more of the design criteria than the initial prototype.

3.4.4.4 Type 1 MkIII

Changes

?? Perspex sides were added to direct the flow of the attractant toward the entrance of the device.

?? A trapezium shape was cut out from the base of the entry door large enough for much of a possum’s head including the nose and eyes, to fit through.

Observations

Possums would follow the scent along the bottom of the perspex plate to the end of the vaccinator. The gap in the door was sniffed with greater intensity than the sides of the device, presumably because this was where the scent was strongest. Although possums frequently sniffed at the door, it was not touched during the observation period.

Conclusion

The shaped cut outs were designed to achieve two goals. Firstly, they would make entry into the vaccinator easier by enabling possums to push on the door with the less sensitive area of the cheeks, rather than the nose. This goal was not achieved. The second goal was to direct possums to the entrance of the device by focusing the scent of the attractant out through the entry door. This goal was achieved.

Reports from other researchers (Short, pers. comm., 1998) indicate that possums would repeatedly use a swinging door, hinged from the roof, as used in the first three vaccinator designs. In contrast to this information, captive possums in this study showed a strong aversion to pushing on any type of flat barrier. Consequently development of the Type One line and the decision was made to generate a completely new design.

3.4.4.5 Type 2 MkI

The Type Two vaccinator was based on a combination of the simplest possible components required to achieve the design criteria. These are an aerosol can, a mechanism by which the possum will activate the can and a basic frame on which to mount the components (Figure 21).

Figure 21. Type two, Mk I vaccinator

Changes

?? The frame consisted of a slightly tapered rectangle, 500 mm long with an open entry end 350 mm square and a closed end of 240 mm square. The floor, sides and closed end were covered in wire mesh.

?? The hinge point of a pressure plate was located 200 mm from the closed end of the device. The pressure plate was connected by wire to the trigger of the aerosol can.

?? The aerosol can was mounted in the top left corner above the pressure plate in a horizontal position. The spray was directed through the wire mesh and out of the device such that it would not contact the possum.

?? Attractant was located under the pressure plate at the back of the device.

Observations

Investigation was thorough though limited to the most dominant possum. After circling the device twice the possum approached the entrance and moved inside. Within the first hour this possum had discharged the aerosol can four times. This possum did not permitted any others to approach the device during the observation period. When the can was first discharged the possum triggering the device showed a strong fright reaction, including a sudden jump and rapid escape. Three nearby possums showed a similar reaction. During the third and fourth discharges of the can the degree of fright shown by the triggering possum and nearby possums was greatly reduced. On three occasions the possum did not walk far enough onto the pressure plate to discharge the can.

Conclusion

This device was attractive to possums and easy to use while also being extremely simple. It essentially satisfies three of the design criteria. The criteria of spray application to the possum may now be addressed, after confirming that the device is attractive to possums and easily used.

Increasing the length of the pressure plate would allow the possum to move further on to it, thereby increasing the force exerted on the nozzle of the can.

Discharge of the can caused a similar degree of fright in possums close to the vaccinator and the possum inside the device. This indicated that either the sound or smell of the discharge was frightening to possums, rather than movement of the pressure plate.

There now appeared to be two areas on which to focus. One was repositioning the can to direct the jet of spray toward the possum. The second was to devise a way of keeping the possum in the device for long enough for the spray to be inhaled.

3.4.4.6 Type 2 MkII

Changes

?? A mounting frame for the can was made and attached to the back wall of the device. When the can was attached to the frame, the nozzle aimed directly at the head of a possum on the pressure plate.

?? The most difficult change was to convert the vertical pull of the pressure plate into a horizontal force required to activate the can when in a vertical position. A very light steel cable (2.5 mm thick) was used to change the force from vertical to horizontal. This passed vertically up from the pressure plate, through a 90 degree angle about a pivot point, then horizontally to the valve.

?? A six way adjustable hinge point for the pressure plate was added to vary the position of the plate with respect to the nozzle of the can and vary the force exerted on the can nozzle.

?? The pressure plate was lengthened by 30 mm to 150 mm.

?? Between 100 mm to 200 mm from the rear of the device additional taper was added to more accurately guide the possum underneath the can (Figure 22).

Figure 22. Type two, MkII vaccinator showing taper above the pressure plate

Observations

This model delivered a dose of spray to the back of the head and shoulders of the possum. However, the angle through which the triggering cable passed generated excessive friction. The result of this was erratic discharge of the can. In addition, after the possum had left the pressure plate the can would continue to discharge as normal for up to five seconds, then discharge as a small leak for a further 30 seconds.

Heavy and light possums would trigger the can at different positions on the pressure plate. In extreme cases, a heavy possum would not have moved under the nozzle before it discharged and light possums would fail to set the device off at all. This problem is compounded by the weight and flex inherent in the pressure plate.

Two of fourteen possums were sprayed.

Conclusion

The basic arrangement of components in this model worked during testing by hand, but observations showed the need for further refinement.

By this stage the important requirements of vaccinator design were beginning to emerge. Primarily, a design was needed where the possum would encounter and accept the various novel parts individually, as it moved into the device. For example, the basic frame and entrance are encountered during initial investigation. Entry allows acceptance of the roof and walls before encountering the pressure plate, taper to direct the head, and finally the nozzle of the can. The gradual presentation of components reduced the degree of fear during investigation. As a consequence there was more chance of the possum investigating the device and less chance of it backing out before activating the can.

A second requirement was that the proportion of weight contributed by the possum to set the device off, relative to the weight of the pressure plate and triggering system, must be as great as possible. As this proportion increases, the range across which the leverage ratio of the triggering mechanism can be manipulated also increases. Essentially this allows more accurate adjustment to ensure the device is triggered by the widest possible weight range of possums.

A third requirement was to minimise flex in the pressure plate, triggering mechanism and can holder as much as possible. Minimising flex was necessary to accurately identify the types and magnitudes of forces operating during activation of the device. On the basis of this, a new design for the trigger was required which was not cable operated. There was also a need for a lighter, more rigid pressure plate.

3.4.4.7 Type 3 Mk I

The basic design of the Type Three was a tapered rectangle, similar to Type Two, but slightly smaller. The interior of the Type Three was divided into a front and rear chamber. The front chamber included the entry, pressure plate and taper to direct the head. The rear chamber provided an empty space and position to mount an attractant container. The purpose of the empty space was to reduce the degree of confinement imposed on a possum as it moved further into the device, while still guiding the head under the can nozzle. Confinement caused apprehension and subsequent avoidance in some possums during earlier observations. Attachment of a container for attractant encouraged possums to move across the pressure plate until the device was triggered.

Changes

- The aerosol can was positioned above the head of the possum to more accurately target the head and shoulders of the possum.
- Size of the entry was reduced from 350 mm square to 300 mm.
- The hinge point of the pressure plate was moved slightly closer (15 mm) to the entrance.
- Space between the pressure plate and rear wall was increased.
- The tapering to direct the possum’s head was moved slightly closer (15 mm) to the entrance.
- The opening between the taper for directing the head of the possum was enlarged slightly.
- Activation mechanism:
- The new activation mechanism was basically a lever and fulcrum (see-saw) design. Force was exerted on one end of the lever by the weight of the possum and pressure plate. The force was transmitted across the fulcrum to the other side of the ‘see-saw’ which acted on the nozzle of the can. Lever length on the nozzle side of the fulcrum was shorter than the pressure plate side, this reduced the degree of movement but increased the force applied to the nozzle of the can.
- A light spring was soldered on to the framing between the pressure plate and the floor of the device. This was to offset the weight of the pressure plate which previously rested on the nozzle of the can via the trigger.

Observations

The reduced entrance size had no effect on the ease with which possums entered the device. Possums moved up onto, and further across, the pressure plate with less hesitation. The spring rate was correct and the lever and fulcrum concept was effective. Overall, the trigger mechanism was a success.

Small notches had been worn on the nozzle of the can causing the trigger to tilt the nozzle in a series of short, sharp movements, causing erratic discharge of the can. There was a small amount of flex evident in the mounting frame for the aerosol can. This greatly complicated setting the leverage force required by the can.

The vaccinator was triggered three times by one possum and once by a second. A third possum triggered the device without being inside, by treading on the edge of pressure plate which extends out through the side of the cage. Discharge of the can caused the individual inside the device and all possums within 1.5 m to flee. However, on two occasions, the first possum to return and re-enter the device was the one which had previously been sprayed.

One possum was particularly aggressive and on two occasions worked either a paw or his nose under the side of the device and then lifted upwards tipping it over.

Six of fourteen possums were sprayed.

When left in the pen overnight, the vaccinator was moved approximately five metres down a gentle slope (probably rolled) by investigating possums.

Conclusion

Attractiveness to possums was not affected by the reduction in size of the device.

The reason possums moved more easily onto and further up the pressure plate appeared to be because they were not approaching the back wall so closely. The apparent possibility of moving into the second chamber lets them move under the valve more easily. Possums were unable to enter the second chamber because they must first cross the pressure plate, causing a discharge of spray which frightened them out of the device.

Additional support of the can holding mechanism and trigger were required to reduce the amount of flex during can activation. To fulfil the criteria of robustness an anchoring system was required to keep the device upright and stationary.

3.4.4.8 Type 3 MkII

Changes

?? The mounting frame for the aerosol can was strengthened.

?? The pressure plate end of the trigger arm was lengthened slightly to improve leverage.

?? The wire loop attaching the trigger to the nozzle of the can was removed.

?? A small plate was added to the end of the lever system to increase surface contact of the trigger with the nozzle.

?? The spring was moved from under the pressure plate and re-attached from the trigger lever to the vaccinator roof. In this position the spring would still offset the weight of the pressure plate and also allow the resting position of the trigger to be set to minimise the resting force on the nozzle of the can (Figure 23).

?? Lateral (undesirable) movement of the triggering lever was reduced by confining movement at the fulcrum.

?? Peg holes were added to the corners of the vaccinator enabling it to be anchored to the ground.

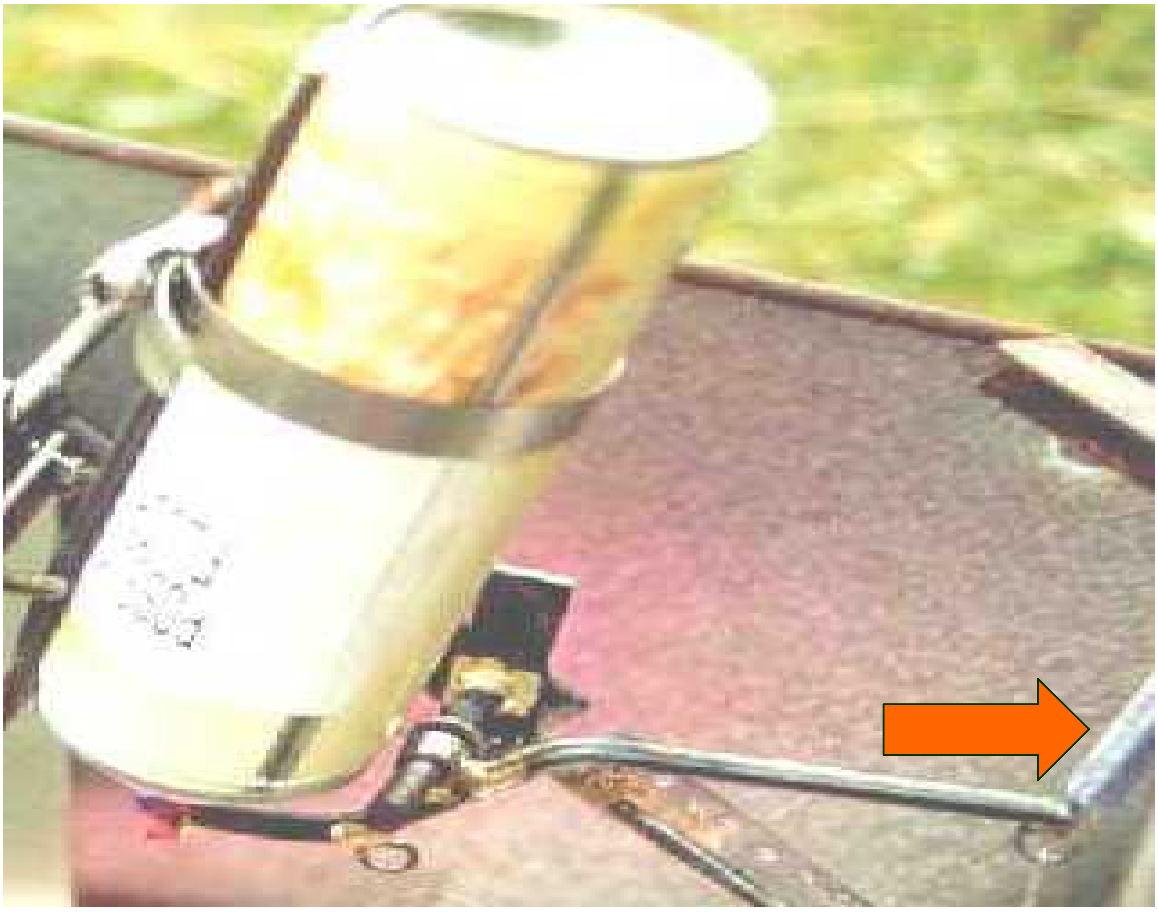


Figure 23. Trigger mechanism and spring (arrowed) on Type three MkII vaccinator

Observations

The unusually aggressive possum was first to investigate. Investigation entailed grasping and shaking the whole device, which was pegged down.

Possums regularly gathered at the back of the vaccinator.

There was more space than necessary for a possum to move in the first chamber.

This group of possums had now been exposed to three models of vaccinator and two in particular were becoming adept at stealing the attractant.

Some possums were seen to chew, or grab and wrench the trigger and nozzle.

Six of fourteen possums were sprayed.

Conclusion

The scent of the attractant should be directed through the entrance of the device.

The size of the first chamber could be reduced slightly. It was good for orienting the possum toward the pressure plate, but this function was now effectively performed by the additional taper between the two chambers.

Some form of attractant container was required. It should be fixed close to the roof to guide the possum’s face as close to the can nozzle as possible. An easy method of access to this container must also be considered.

Some form of protective structure was required around the trigger and can nozzle to stop damage caused by investigating possums, particularly to the soft, plastic, removable nozzle.

3.4.4.9 Type 3 MkIII

Changes

- The overall length was reduced from 500 mm to 360 mm, with the first chamber shortened by 120 mm.
- The height of the vaccinator was reduced from 300 mm to 280 mm.
- A perforated attractant container was added. It measured 80 x 100 mm and 45 mm deep and attached to the back wall, just below the roof. A small hatch in the roof of the vaccinator provided easy access to the container.

?? The pressure plate was lengthened by 10 mm. The end opposite the hinge was bent up slightly to discourage larger animals from stepping over the plate into the second chamber.

?? Six shaped perspex plates enclosed the rear chamber, replacing the wire mesh. Silicon putty was used to seal the joins between plates. Attractant could now escape only across the pressure plate and through the entrance of the device.

?? The perspex would also function as protection for the trigger, but did not protect the nozzle.

?? This model was initially tested without an aerosol can in order to record the behaviour of possums when no frightening stimulus resulted from stepping on the pressure plate.

Observations

Possums would actively explore all parts of the vaccinator interior in search of the attractant. Movement of the pressure plate caused by the weight of the possum did not cause alarm. The attractant container was still accessed by the aggressive possum at the end of the observation period.

An investigating possum was forced to arch its back sharply in order to reach the attractant container, indicating that the container required relocation in a more accessible position.

Six of fourteen possums were sprayed.

Conclusion

The discharging can was clearly responsible for the fright shown by an investigating possum when the vaccinator was triggered. Previous observations had shown the sound of the can discharging, rather than the stream of spray, caused the most vigorous reaction in possums.

The frightening effect of the can was important in ensuring a possum left the vaccinator before it could explore and possibly damage the moving parts.

Some modifications were required to the attractant container to make it more approachable and secure.

3.4.4.10 Type 3, MkIV

Changes

?? The trigger mechanism was changed to operate along the midline of the device. Previously it was approximately 45 degrees off the midline.

?? A 40 mm square plate was used to mount a fulcrum for the trigger. This allowed excellent adjustment of the shaft to find the position where the trigger worked most effectively.

?? The point where the attractant container attached was shifted to the midline of the vaccinator and lowered slightly.

Observations

A new colony of possums were used in the first observation period and their investigation was centred on the general pen environment rather than the vaccinator. Twelve of the fourteen possums approached the vaccinator entrance, although it the was activated less frequently than in previous sessions. Possums would frequently approach and briefly step on to the pressure plate, but then back out of the device, without triggering the can. From this, it was assumed that the opening from the end of the first chamber into the second was too small. The framing to which the trigger attached was also making the opening seem small.

The attention of possums was attracted to the trigger mechanism and can nozzle, rather than directed toward the attractant further inside.

Two of fourteen possums were sprayed.

Conclusion

The opening between the two chambers required enlargement, both horizontally by manipulating the framing used to mount the trigger, and vertically by raising the position of the can and trigger. Presented with a larger intrance, possums should move more freely on to the pressure plate to activate the spray.

3.4.4.11 Type 3 MkV

Changes

?? The plate, on which the trigger was mounted, was reduced in size.

?? The trigger mechanism, can and holder were all raised 8mm.

?? The width of the entrance to the second chamber was also increased slightly (8mm).

?? ‘Aerozone’ aerosol cans were replaced with specifically made cans containing four dye colours, made by Aerosol Products, Auckland, New Zealand. Cans containing gentian violet dye were used for the remaining pen trials.

Observations

Possums were much less apprehensive about moving toward the attractant across the pressure plate. The opening between the two chambers would appear larger if the plate to which the trigger attached was removed and the shaft attached permanently.

Gentian violet dye is an excellent marker. However effectiveness of the other three colours, which are paint based, rather than a dye, was poor.

Conclusion

The changes were effective at enlarging the opening to the second chamber, but this area would ideally be enlarged further still.

It was now relevant to begin thinking about the implications of making a number of copies of a vaccinator for trial in the field. Some adjustment would be required to compensate for the small differences in replicates. Incorporating adjustment into the attachment point of the can would allow the fine tuning of duplicates without the need to manipulate the intricate triggering mechanism.

The fulcrum of the trigger was found to be most effective when placed as close to the aerosol can as possible and 25 mm above the nozzle.

Gentian violet was the most effective dye for marking possums.

3.4.4.12 Type 3 MkVI

Changes

?? The new can holding device was based on a 250 mm length of 20 mm x 3 mm plate which was bent to a V shape. The aerosol can was clamped to one arm of the V, while the other arm was clamped to the roof of the vaccinator.

?? A strong clamp was welded to the roof of the vaccinator.

?? Some modification of the roof was required to accept the new can holding design.

?? An electrical double plug box replaced the hand made bait container. It was an ideal size, easy to attach and coarsely perforated.

?? The plate used to fine tune placement of the trigger pivot shaft was removed.

Figure 24. Can holding mechanism showing V shaped steel plate and clamp (arrowed)

Observations

The enlarged opening to the second chamber, caused by the new can holding mechanism, improved possum access. Adjustability of the V shaped can mount allowed the contact between nozzle and trigger to be optimised. The nozzle of the can could be adjusted in any direction to suit the trigger. The new attractant container was possum proof.

Figure 25. The final stage of development for the prototype vaccinator, Type 3, Mk VI

Conclusion

The current vaccinator (Figure 25) fulfilled the five design criteria. It was attractive to possums and could be easily used. Marking the possums with dye was achieved by directing the spray toward the back of the head and shoulders of the possum. The device was very simple and used only a lever and spring to dispense the spray. It was also robust, could be firmly attached to the ground and was adjustable to allow fine tuning of operation.

At this point, 13 copies of the Type Three, MkVI vaccinator (Figure 25. The final stage of development for the prototype vaccinator, Type 3, Mk VI) were constructed.

3.4.4.13 Type 3, MkVII (Steel copies)

Three steel copies constructed by Art In Iron (Palmerston North, New Zealand) were of almost identical design to the prototype. Minimal modifications were required before they were suitable for field trials.

The ten copies of the final vaccinator design presented by Iron Village (Palmerston North, New Zealand) incorporated a very different trigger mechanism and can holding mechanism (Figure 26). Initially these devices would work effectively, although there was doubt about robustness and the degree of flex during operation, which was much higher than the prototype.

Robustness of the replicates was tested during a preliminary field trial. The amount of use by possums was not investigated during this trial. After four nights only four of thirteen vaccinators were working properly, one of which was the prototype. All copies were returned for modification.

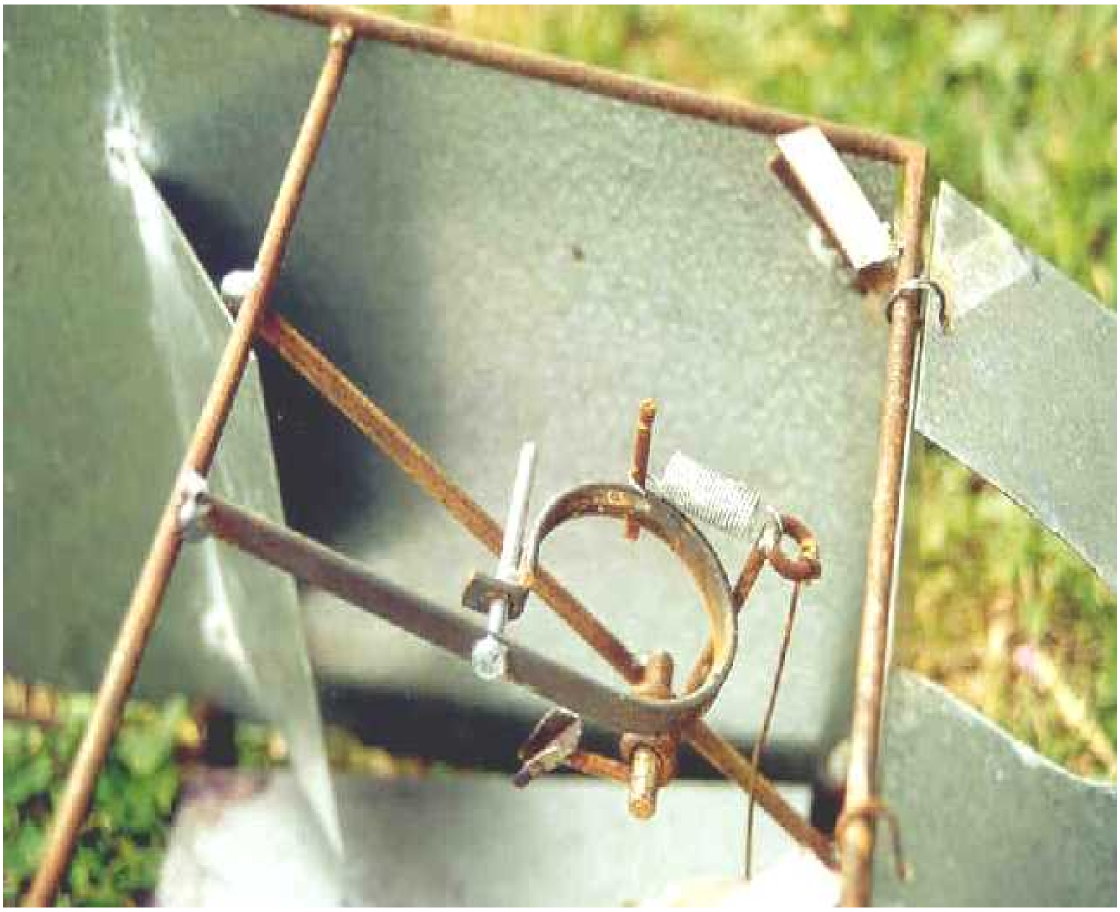


Figure 26. The can holding and triggering mechanism used in the twelve copies of the final vaccinator prototype

Changes

?? The steel pressure plate was replaced with an aluminium copy.

?? The can holding mechanism was replaced with the prototype V design.

?? The trigger mechanism was replaced with the prototype version of the lever and fulcrum design.

?? Lighter springs were used to suit the reduced weight of moving parts.

Observations

Observations using captive possums showed that modifications had greatly improved the rigidity of the device. Can movement was minimal and adjustment improved. The weight of moving parts was also reduced. The trigger and can nozzle were much more effectively protected. Eight of fourteen possums were sprayed.

Figure 27. One of twelve steel copies (type 3, Mk VII) of the final vaccinator design after the changes listed in section 4.4.13

Conclusion

The vaccinators were now virtually identical to the prototype. Operation of a sample of three copies was satisfactory and all copies were now prepared for the first genuine field trial.

3.4.4.14 Type 4 MkI (Wooden duplicates)

Two duplicates of the final steel prototype were constructed from a combination of wood and plastic (Figure 28). The aim of the copies was to compare behaviour of possums toward vaccinators made from different materials and of different appearance. As with the steel copies, some modifications were required, to achieve an operating standard equivalent to that of the prototype, before pen trials could commence.

Changes

?? The trigger fulcrum replaced with a stronger steel model to reduce flex.

?? The trigger was extended at the pressure plate end to increase leverage.

?? The wooden can holder was replaced with a V shaped can holder to reduce flex.

?? A spring was attached from the pressure plate end of the trigger to the roof as used on the steel prototype.

?? The steel pressure plate was replaced with an aluminium copy.

?? The trigger mechanism presented worked at 90 degrees to the midline (across the vaccinator). It was modified to operated along the midline of the device.

Observations

Eight possums were found to approach and enter, or attempt to enter the wooden model compared with twelve for the steel one. The steel device has less flex, improving the accuracy of fine tuning. Small additions and subtractions to vaccinator design, or adjustment of angles is more easily accomplished with steel than with wood. Disassembly of the entire unit is required to access some components of the wooden model, for example to enlarge the opening into the second chamber. Screw holes fixing wooden parts have increased flex when the device is reassembled. Wood will swell, contract and warp in response to environmental conditions.

Three possums were observed chewing the wooden sides. No possums were sprayed with dye.

Figure 28. One of two wooden vaccinators after the changes listed in section 4.4.14

Conclusion

The main conclusion was that wooden models appeared less attractive to possums than their steel counterparts. In addition wood, as a construction material, had several qualities which were undesirable for the precise production, attachment and fine tuning of small moving parts required by a vaccinator.

3.5. DISCUSSION OF PEN TRIALS

A total of 75 hours were spent observing the investigative behaviour and social hierarchy of four captive possum colonies. A series of novel objects were presented first, followed by fourteen variations of vaccinator. The majority of captive possums were found to be naturally curious animals and to display minimal avoidance behaviour toward novel objects. It was found that the best period for watching was from when possums first emerged in the evening until the end of their first active period, which spanned approximately three hours. During this time possums were comparatively energetic while feeding and grooming. They would also pay more attention to novel objects than after approximately midnight. The pattern of an activity period immediately after waking followed by a rest period and then another activity period before dawn has previously been reported by Cowan and Clout (2000).

Neophobia

The objective of investigating novel objects is to provide an animal with species preserving knowledge about the world (Glickman and Sroges, 1964). The approach and investigation of a novel object by an animal is driven by a combination of curiosity and fear. The degree of attraction shown by an animal species toward an object varies between order, family and even individuals.

In a pen situation the majority of captive possums displayed minimal neophobia toward a range of introduced objects. A similar result was found when comparing the investigative behaviour of possums with 29 other mammals (Russell and Pearce, 1971). However a small proportion in each of the four observed possum groups would not approach any novel object. This variability between individuals has been previously identified in a study of trappability of wild possums by Green and Coleman (1981).

In some cases, groups of possums would frequently investigate an object together, indicating indifference to the presence of conspecifics during investigation. However, in other cases, a subordinate individual may wait for several hours before it could approach the object, without fear of being disturbed. There were no differences between the investigative behaviour of male and female possums, consistent with a study of mature pairs (male and female) of 53 species including marsupials, primates, carnivores, reptiles and rodents (Glickman and Sroges, 1964).

The method of investigation by possums was based primarily on smell and taste, with sight and sound playing a smaller role. This is similar to the results of Glickman and Sroges (1964) who found taste to be the main form of investigation of over 100 tested species including marsupials, primates, carnivores, reptiles and rodents. Novel objects were examined thoroughly by most possums in the current study. The majority of possums would attempt to enter hollow objects and small spaces in search of an attractant. These observations are supported by Carey *et al.* (1997) and Short (pers comm., 1998) who found that possums were naturally curious and would actively investigate novel objects.

Addition of an attractant was important in maintaining the attention of possums. When no attractant was present a brief investigation was conducted and the object was then ignored. In contrast, one particularly aggressive male was seen to chew the wire mesh and tip over some models of vaccinator while searching for an attractant. A multitude of attractants have been used to attract possums to traps. The use of an attractant has been shown to increase trap catch rate of wild possums in an experimental trial by Cowan (1987). However, formal comparisons have struggled to find significant differences in the effectiveness of various compounds and even differences between attractants and water (Morgan *et al.*, 1995).

Design path of an aerosol vaccinator

The first prototype vaccinator was designed in consultation with experienced possum trap designers and researchers. However pen trials showed the device to be a dismal failure. It became clear that, despite several modifications, the concept of swinging doors could not be incorporated into a device designed for use by possums.

To facilitate a new approach, the fundamental components of an aerosol vaccinating device were defined. These were an aerosol can, a way of using the movements and body weight of the investigating possum to discharge the can, a way of attracting and maintaining the attention of the possum, a means of containing the possum and a frame to hold the components together. These concepts were incorporated into the Type Two vaccinator and gradually refined to the point where the device fulfilled the design criteria.

During the design process it became clear that there are three key principles which determined how effectively a specific model of vaccinator would fulfil the design criteria. The first of these principles was presentation of one component, or feature of the device at a time. A possum entering a vaccinator in search of the attractant first encounters the confining sides of the device. After moving a short distance inside, it then encounters the beginnings of the taper. After moving a further short distance the possum discovers, and is required to step on, a pressure plate. More possums discharged the vaccinator when each component was presented in sequence, compared with early designs when they were presented simultaneously.

The second principle was maximising the force generated by the trigger system, so as to effectively discharge the aerosol can. The magnitude of the force was determined primarily by the length of the lever acting as the trigger and the position of the fulcrum. It was also determined by the position of the possum on the pressure plate. In addition, it was vital to minimise the amount of flex in all moving parts so as to exert maximum force on the nozzle of the can.

The third principle was in fully utilizing the weight of the possum as the triggering force. This was achieved by keeping all parts of the triggering system, primarily the pressure plate, as light as possible. The result was effective triggering of the device by possums, covering a wide range of body weights.

The final model in the development process met the five design criteria. Firstly attractiveness to most possums was achieved by using an olfactory lure, and design features of the device which made it easily accessable. Secondly it was easily triggered by possums, as they stepped on a pressure plate. The aerosol can was discharged by the weight of the possum and the target area of the stream of spray was easily adjustable. The can was also easily accessable. The fourth design criteria was overall simplicity. There was no requirement for an external power source, the device was adjustable in several ways and had only two moving parts. Finally, to provide a robust design, peg points allowed it to be firmly anchored to the ground and the few moving parts were protected from damage during handling or investigation by possums.

The noise of the aerosol can being discharged was the strongest deterrent feature of the vaccinator to investigating possums. However, possums quickly learned that the sound was harmless and in some cases the possum retreated only so far as to avoid the stream of spray.

Social hierarchy

The dominant animal would be first to investigate a novel object or vaccinator during which it would defend the device against the approaches of other possums until its inspection was complete. Following this, individuals in the middle social order were permitted to investigate the object, frequently in a group of up to six. Within the immediate vicinity of a novel object fights were rare, with most agonistic interactions settled by an aggressive posture or vocalisation. The incidence of aggressive response to aggressive behaviour has also been noted as low in other marsupials (Russell, 1970). It has been previously noted that possums mark areas or objects of interest with scent glands and urine. The effect of this was to accord the marker exclusive rights to the marked area (Kean, 1967). During the pen trials scent marking of several was frequently observed, however no novel objects or vaccinators were marked in this way. Over the duration of an observation period all possums which showed interest in an object would eventually investigate it, either in a group or as individuals after the group had dispersed.

A small proportion of each colony would not approach any novel object and this may have been for several reasons. Firstly, these individuals may have adapted poorly to captivity. Characteristic stress behaviour included long periods spent circling the perimeter of the cage, or huddled in groups in dark corners. Secondly, failure to approach the presented objects may also have been caused by a strong fear of novel objects, or the presence of humans, or the artificial light used during observation. It is also possible that the area of the pen was sub-divided into ranges, and that the range of some possums did not include the observation area. A number of possums moved throughout the whole pen, however these were almost always animals in category one, high in the social order. Subordinate possums would avoid confrontation with dominant individuals. However, the division of a pen into any form of range has not been reported previously in possums.

The social interactions observed around novel objects and vaccinator designs was very similar to that observed in both wild (Winter, 1976) and other captive (Biggins and Overstreet, 1978) possum populations. In the current study, colonies of either males or females had one dominant animal. Females appeared to assert dominance more aggressively, as previously reported by Hickling *et al.* cited in (Day *et al.*, 1998). Below the dominant animal, the middle order had no clear ranking and the outcome of agonistic interactions between these members was dependent on circumstances specific to that incident, for example the strength of position of each individual during the encounter. In two of four possum groups observed, there was one individual that would frequently sniff noses and share the food of other possums. Both nose to nose sniffing and food sharing have previously been described as affiliative behaviour, characteristic of subordinate animals (Winter, 1976). It is concluded that these two possums were at the bottom of the social order. Observations of affiliative behaviour in this study were rare in comparison with pen trials of both male and female possums by Day *et al.* (2000). The trend of a single dominant and subordinate animal, with a mixed middle order has also been noted in possums by Biggins and Overstreet (1978) and in the grey kangaroo (Grant, 1973). The size and weight of a possum did not appear to have any influence on its position in the social order. Interactions would usually begin when the individual space of two possums overlapped. This space had a radius of approximately one metre as previously reported by Oldham (1986) and Day (1996).

Overall, it was concluded that a social order exists in captive possum colonies. It was characterised by a single dominant animal, a largely changeable middle order and in some cases one individual at the bottom of the order. The social order did not influence the proportion of a colony which would investigate a novel object. However, this assumption may be incorrect if the small proportion that would not approach an object were subordinate possums and as such, restricted by dominant possums to an area of the pen remote from the object or vaccinator.

The effect of captivity on possum behaviour

It is difficult to estimate the effects of captivity on the investigative behaviour of possums. There are a number of reasons why the behaviour of captive possums may be different from those in the wild. The population density was much higher in captivity than that found in the wild, causing a higher degree of social interaction than is found in this normally solitary animal. Despite this, all possums attracted to a novel object were permitted to investigate it at some stage and very few fights were observed.

Captive possums expend no time or energy on finding food, as fresh fruit and vegetables were delivered daily. It is possible that this energy was instead spent on investigation of novel objects, making investigation more intensive than that shown by wild possums. Modification of sleeping habits was evident in some possums that became active during daylight in association with the delivery of food to the pens. Fear of humans was also greatly reduced and it seemed that the presence of humans become increasingly associated with food. If the possum associates objects presented by humans with food then investigation of a novel object may have been more intense on the premise that food would be found in it somewhere.

In each possum colony the investigation of some individuals became progressively less wary over a number of observation periods. This conditioning may not be shown in wild possums. In addition some captive possums successfully recovered the attractant on occasion. The experience of receiving a reward as a result of investigating an object would encourage more active inspection than the experience of being frightened by the sound and spray from a discharging aerosol can.

As possums were caged in single sex groups the influence of between sex interactions could not be observed. However, the investigatory behaviour observed was very similar between sexes.

It is clear that there was a gradual acclimatisation to captivity. Behaviour of one colony on the night of their introduction to the pen was very different to that seen in the same group two weeks later. During the trials, most possums were not visibly stressed after two weeks or more in the pen.

Part two: Field trials of an aerosol vaccinator

3.6. INTRODUCTION

Pen trials have provided the first three stages in the development of an aerosol vaccinator. Presentation of various novel objects showed that most possums exhibit minimal neophobia and will actively investigate novel objects. Observing social interactions in four different groups has shown that a social hierarchy exists, but has little effect on the proportion of the colony which investigate a novel object. The progressive development of the vaccinator was based on the results of subjective observations made during presentation of thirteen distinct models of the device. Development concluded when the device had achieved the design criteria. Following the construction of thirteen copies of the final design, planning began for the first of five field trials.

3.7. MATERIALS AND METHODS

3.7.1 Site description

Trials were conducted at the Castlepoint study site in the Wairarapa, New Zealand which is an area of 56 hectares, covered predominantly in scrub but with some areas of improved pasture (40? 51’ S, 176? 14’ E). A more thorough description is presented in the materials and methods section of the Chapter 2.

On the site were 450 cage traps which were set for three consecutive nights at bimonthly intervals to examine the possum population. The live trapping study was based on a capture-tag/release-recapture program and used to monitor possum population dynamics and incidence of tuberculosis.

3.7.2 Trial description

Five trials were conducted between March 1999 and January 2000. Each trial was slightly different and ranged from three to fifteen nights. The basic procedure for all trials remained the same. Vaccinators were set at fixed sites to provide the most effective cover of the study site, given the number of devices. During the first four trials with five vaccinators, density was 1/11 hectares (ha). In the final trial with fourteen vaccinators the density was 1/4 ha.

Three types of vaccinator were used during the trials. These were the prototype, thirteen steel copies (ten supplied by Iron Village, Palmerston North, New Zealand and three supplied by Art In Iron, Palmerston North, New Zealand) and two wooden copies.

Three types of attractant were used. These were approximately fifteen grams of chocolate, apple segments dusted with cinnamon and a 4 cm cube of foam rubber impregnated with 0.5 ml of cinnamon oil (Quest International, Auckland, New Zealand).

Bait stations were mounted within two metres of each vaccinator. At the beginning of trial number three and number five bait stations were filled with cereal based possum pellets. During the other three trials, bait stations were not used. Aerosol cans containing gentian violet and water based paint type dyes (Aerosol Products, Auckland, New Zealand) were used in steel and wooden vaccinators respectively. Cans were positioned to spray dye on the back of the head and shoulders of the possum.

A check of each vaccinator site and the operation of the device was carried out after seven nights during trials which ran for longer than one week. The attractant was also replaced during these checks. The duration of a trial was measured in ‘vaccinator nights’. The total number of vaccinator nights was equivalent to the number of vaccinators multiplied by the number of nights for which they were set.

Cage traps already present on the site were used to check possums for dye by two methods. The first method was to set all traps within a 150 m of vaccinator sites for two consecutive nights. Examination in this case consisted of examination of trapped, non-sedated possums for signs of dye by parting the fur from the head down the back on both sides of the body. The second method coincided with the bimonthly examination of the population where all 450 traps were set for three consecutive nights. Captured possums were thoroughly checked for signs of dye while sedated with ketamine (1 ml, 10 g/ml, Parnell Laboratories New Zealand Ltd, 233 Porchester Road, Takanini, New Zealand).

Jolly-Seber estimates of the adult possum population were generated using bimonthly trapping information. These estimates were used to calculate the proportion of the possum population marked by dye during each trial.

3.8. RESULTS

3.8.1 Field trial one – trapping for dye only

The first field trial began in March 1999 and involved five vaccinators, the original prototype, two wooden copies and two steel copies. This gave a density of one vaccinator/11 ha. Chocolate was used as the attractant. Distribution of the five sites relative to possum density is shown in Figure 29.

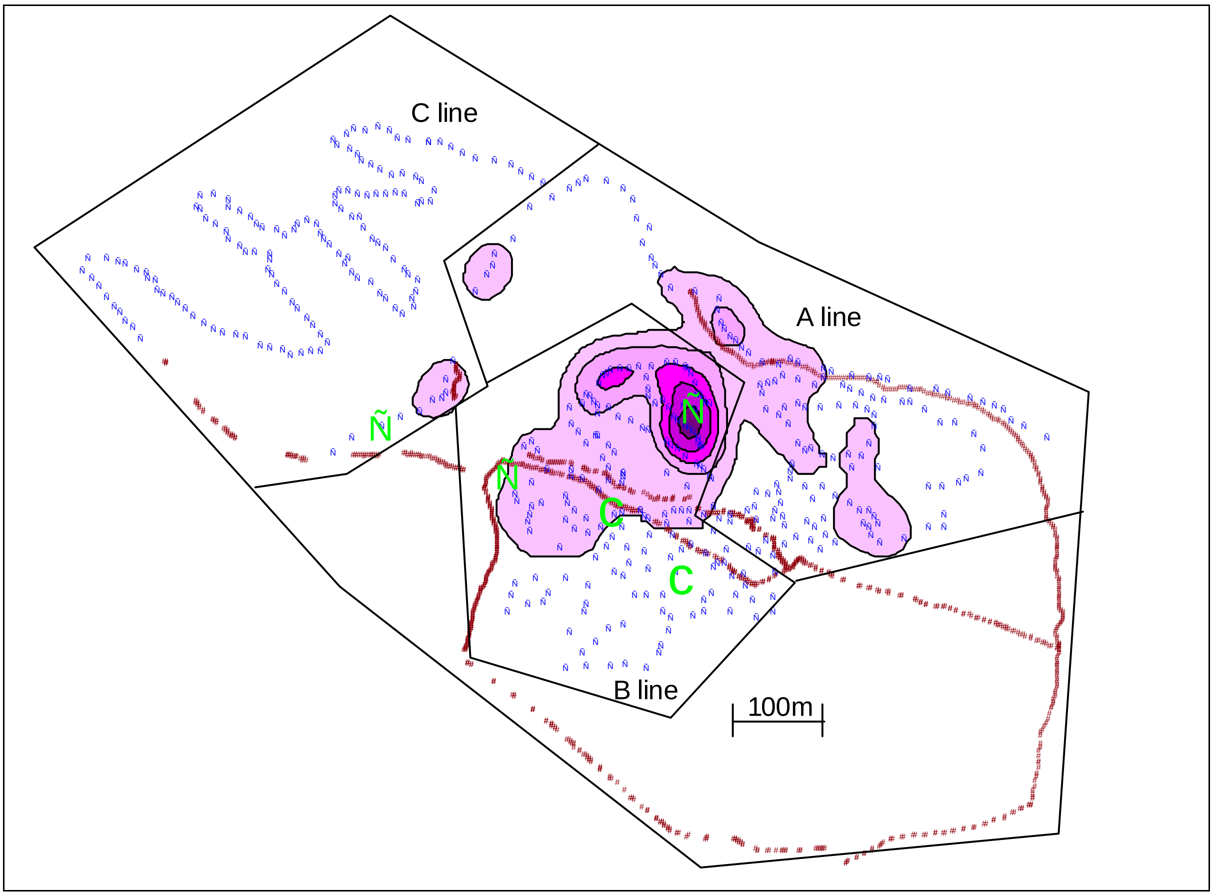


Figure 29. Vaccinator distribution on the Castlepoint study site during field trials 1–4. Large crosses represent steel vaccinator sites, flags represent wooden vaccinator sites, small crosses represent trap sites. Contours represent possum density, as shown by 985 live trapping events for 30 possums, from high density (most frequently used 20% of trap sites, darkest shade), through 40%, 60%, to low density (80% of used sites, lightest shade). Thin line represents the boundary of the study site and areas of the three trap lines, thick line represents fence lines

A check was carried out after seven nights. All vaccinators were functioning acceptably and the attractant was replaced. After 40 vaccinator-nights all cage traps within 150 m of a vaccinator site were set. No captured possums appeared to have been marked with dye. Subsequent vaccinator field trials showed that non-sedated live trapped possums could not be examined closely enough to identify all dyed animals. As a result of this, subsequent field trials were timed to coincide with the bi-monthly examination of the possum population during which all possums were sedated. Field trials using smaller scale trappings (within 150 m of the vaccinators) continued but where used primarily for checking reliability of the vaccinators and levels of possum activity about the vaccinator sites.

3.8.2 Field trial two – August 99 bimonthly trapping

The second field trial in July 1999 also used five vaccinators (Figure 29) set for 45 vaccinator nights using chocolate as an attractant. During the check after seven nights it was found that the action of both wooden vaccinators was adversely affected by wet weather, which caused some of the wooden parts to expand. Eight possums were found marked with purple dye during the bimonthly examination. Purple dye was used in the steel vaccinators while green dye was used in the wooden vaccinators. All possums were marked near the shoulders and the quantity of dye varied between individuals from 0.5 cm^2 to three distinct areas of approximately 2 cm^2. The Jolly-Seber population estimate for the study site using the August trapping data was 87 adult possums. Using this data, nine percent of adult population had been marked with dye.

3.8.3 Field trial three – trapping for dye only

Before the third field trial began both wooden vaccinators were replaced with steel copies at the same site. The five vaccinators were set in August 1999 for 75 vaccinator nights using chocolate as an attractant. Bait stations were installed at vaccinator sites and filled once with possum pellets at the beginning of the trial. After seven nights the operation of each device was checked and attractant replaced. Three possums were found marked with dye after a small scale trapping over two consecutive nights. Using the same population estimate as in trial two, four percent of the adult population had been marked with dye.

3.8.4 Field trial four – October 99 bimonthly trapping

The fourth field trial was timed to coincide with the October bimonthly trapping. The attractant was changed from chocolate to segments of apple dusted with flour and cinnamon. This was the same attractant as used in the cage traps. Attractant in all vaccinators was replaced after five nights. Vaccinators were set for a total of 45 vaccinator nights. Trapping identified twelve dyed possums. The Jolly-Seber estimate for the study population from the October trapping data was

64 adult possums. Using this figure 19% of adult possums had been marked with dye. Three possums were found marked with both bright and faint dye. A check of possum identification numbers showed these possums had been dyed during the previous trial.

At the completion of this trial an additional ten copies of the original prototype were constructed from steel.

3.8.5 Field trial five – depopulation of the study site

The fifth and final trial was carried out over the three nights prior to the start of the study site depopulation. Fourteen vaccinators were used on the five original sites and nine new sites. Bait stations were also present at all sites and filled once at the beginning of the trial. Attractant was identical to that of the fourth trial, but in addition a 4 cm cube of foam rubber, impregnated with 0.5 ml of cinnamon oil was added.

Figure 30 shows the distribution of vaccinators across the study site at a density of 1/4 ha. An effort was made to evenly distribute vaccinators over the study site.

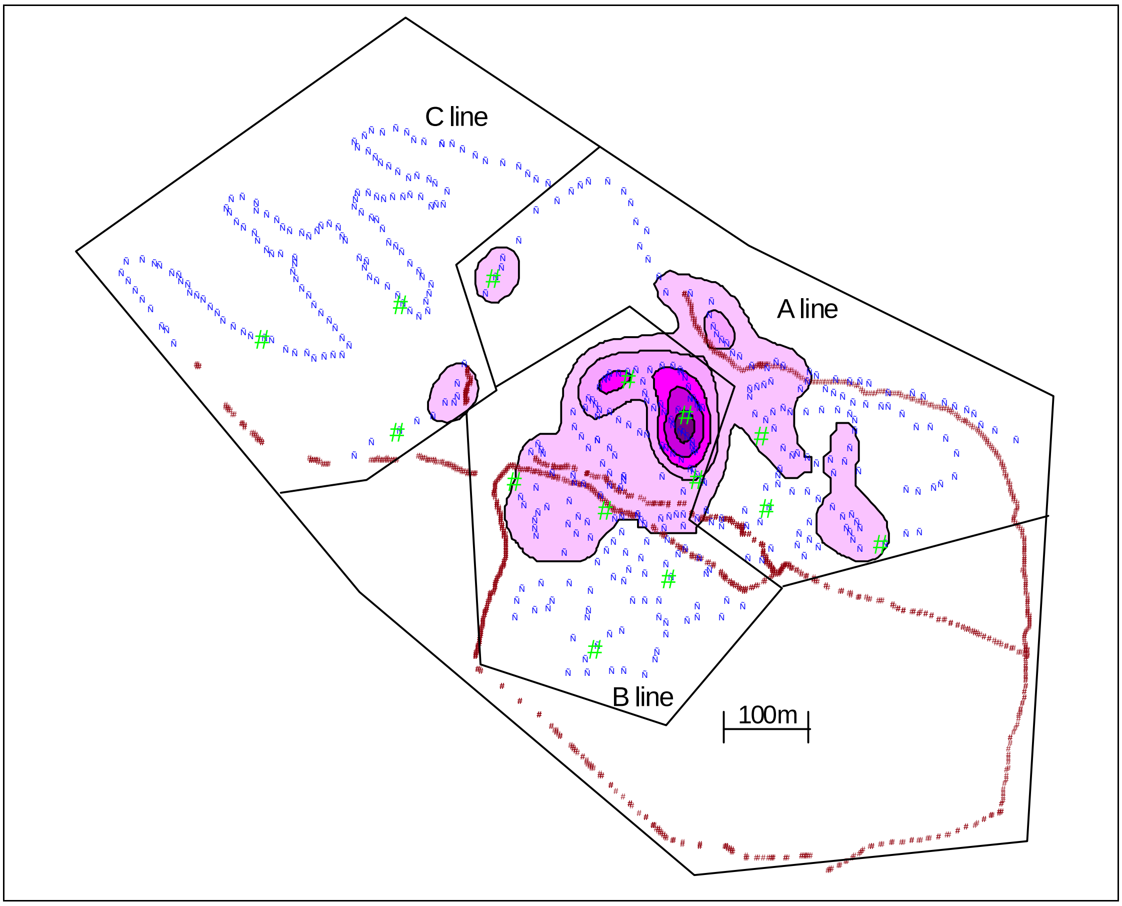


Figure 30. Vaccinator distribution on the study site during the fifth field trial. Crosses represent trap sites, circles represent vaccinators, contours represent possum density, as shown by 985 live trapping events for 30 possums, from high density (most frequently used 20% of trap sites, darkest shade), through 40%, 60%, to low density (80% of used sites, lightest shade). Thin line represents the boundary of the study site and areas of the three trap lines, thick lines represent fence lines

The duration of the final vaccinator trial covered six nights, three before and three after the start of the depopulation, giving a total of 84 vaccinator nights. After the first three nights of the depopulation, the majority of the study population had been trapped. At this point vaccinators had been set for six nights and effectiveness of the attractant was decreasing rapidly. During the following three weeks trap catches decreased markedly and an increasing proportion of the possums caught were non-tagged individuals, presumably from outside the study site. On the first day of the depopulation, using B line traps a total of 27 possums were caught. Sixteen (61%) of these possums were marked with purple dye. On the second day traps on the A and B line were set and four possums were trapped. One of these (25%) was marked by dye. On the third day A, B and C trap lines were set and nine (50%) of 18 trapped possums were marked by dye.

During the depopulation, a total of 126 possums were trapped. Seventy four of these were part of the study population. Fifty two were non-tagged possums considered to have moved onto the study site after the beginning of the depopulation. Eleven hedgehogs were also trapped. A total of 26 possums and one hedgehog had been marked by dye. The Jolly-Seber population estimate predicted the study population to contain 76 adult possums during this period. On the basis of this, 34% of the adult population had been marked with dye.

In some cases individual possums were marked with dye over the shoulders, head, face and parts of the back. There were also visible trails of dye, indicating some possums had been moving around underneath the stream of spray. No dye was found on the tail or back legs, indicating the possum had moved across the pressure plate into the second chamber.

It is estimated that during the depopulation, twelve of the fourteen vaccinators were working properly at any one time. Problems centred around the aerosol can including the loss of one nozzle, dislodging of a trigger from the nozzle and a small leak causing a total loss of pressure.

Vaccinators remained in the field for four months after the depopulation. During this period the pressure drained from four of the cans and one vaccinator had been damaged beyond repair after being stood on by cattle.

3.8.6 Summary

Table 14. Summary of vaccinator field trials

Trial number	1	2	3	4	5	Overall
Number of vaccinators	5	5	5	5	14	average: 6.8
Number of nights	8	9	15	9	6	47
Number of vaccinator nights	40	45	75	45	84	289
Number of checks	1	1	1	1	0	4
Bait station filled	No	No	Yes	No	Yes	twice
Bait used	A	A	A	B	B&C	N/A
Population estimate	95	87	87	64	76	average: 82
Dyed possums	0	8	3	12	26	46*
% of population dyed	0	9	3.5	19	34	56
Trap method	D	E	D	E	F	N/A

A = baited with chocolate, B = baited with cinnamon dusted apple segments, C = baited with cinnamon oil on foam rubber. D = all traps within 150 m radius of a vaccinator were set, E = normal bimonthly examination, using cage traps, F = depopulation, using cage traps and leg hold traps.

* A total of 49 possums were marked with dye, three individuals were marked twice during the trials, but included in this table once

The five field trials were conducted over eight months during which vaccinators were set for a total of 289 vaccinator nights. During the trials 46 individual possums were trapped which had been marked by dye. Three of these were re-marked by dye in subsequent trials. The average of the four population estimates over the eight month period was 82 possums. If 46 of these were marked with dye, then 56% of the study population had been in contact with the aerosol spray at some point during this eight months. It is highly possible that not all possums marked with dye were live trapped for identification.

3.9. Discussion of field trials

Field trials provided a natural environment and a wild possum population with which to test the vaccinator. Trials aimed to identify weaknesses in the various components of the vaccinator design. They also allowed a simple assessment of associated factors such as bait stations, attractant and vaccinator density. Finally a rudimentary estimate of the proportion of a wild possum population which would use the device was calculated.

Preliminary estimates of vaccinator efficiency

During the five field trials vaccinators were set for a total of 289 vaccinator nights over a period of eight months. The proportion of the adult population marked with dye in each individual trial increased from zero to 34%. Due to the manipulation of various factors between trials it is not possible to identify which changes were responsible for the increase in number of dyed possums. Over the eight months at least 46 individual possums were marked with dye, or 56% of the estimated adult population.

Three possums were dyed twice during the field trials. Evidence that possums would repeatedly trigger a vaccinator was important as it was unknown if re-vaccination would be required to maintain a high level of immunity. Possums reaching adulthood are likely to survive between five and seven years (Efford, 2000). The duration of protection with bacille Calmette-Guerin (BCG) vaccine in possums is unknown, although a protective effect was detectable twelve months after administration (Corner, unpublished). Twelve vaccinations at weekly intervals induced the greatest protective response when compared with two doses at a six week interval, and a single dose, however the response to twelve doses was not significantly different from that to a single dose (Corner, unpublished). A study in humans indicated that repeat vaccination with BCG (2 doses) increased the incidence of tuberculosis in comparison with a single vaccination (Rieder, 1996).

A vaccine of less than 100% efficacy and with less than the entire population being vaccinated can be effective because of the effect of herd immunity. The concept of herd immunity states that protection of a proportion of a population against disease will reduce prevalence of disease in the total population, due to the low level of susceptible hosts. It also indicates that when the protected proportion of the population reaches a threshold level, disease can no longer persist due to a lack of susceptible hosts. The factors influencing this threshold are specific to the species and disease in question. The computer model designed by Barlow (pers. comm., 2000) applies the concept of herd immunity to vaccinating wild possums for tuberculosis. The model indicates that when the proportion of a possum population protected against tuberculosis exceeds 54%, the disease can no longer be sustained within that population. A second model (Roberts, 1996) suggests that tuberculosis could be eradicated from possums by maintaining 40% of the population in an immune state, or by vaccinating possums at a rate of 13% per year. This model also suggests that disease would drop to 10% of initial prevalence by vaccinating 13% of the population per year, with no control operations. Unfortunately this model does not take into account some important aspects of possum biology, for example, immigration of young animals. It also assumes that vaccination leads to total protection against disease, for the entire life of the possum.

To put the field trial results in a ‘real world’ perspective, the following theoretical scenario is presented. It is based on the overall dye rate of 56% and two assumptions. Firstly, that the vaccine produces a protective response in 80% of possums sprayed by a vaccinator, because some possums may leave the vaccinator before being exposed to sufficient vaccine to elicit a protective response. Secondly, that the response lasts for a minimum of eight months. Accepting these assumptions, 45% of the adult possum population would be protected after 289 vaccinator nights. Ten vaccinators set for one month (29 nights), over 91 ha, provides an equivalent vaccinator density and duration to that used in the trials. This in turn would theoretically lead to the protection of 45% of the possum population in that area. If re-vaccination after a period, for example eight months, is required, it is possible that some individuals not vaccinated during the first operation would be vaccinated during the second. This would increase the protected proportion of the population. However, it is also possible that some animals vaccinated during their first exposure to a vaccinator may not repeat the process when exposed to the device for a second time.

It is accepted that many factors will potentially affect the hypothetical vaccination scenario presented above and the computer model predictions. However, the aim is to demonstrate two important points. Firstly, it is possible to apply aerosol spray to a considerable proportion of the wild possum population using a vaccinator. Secondly, this proportion is not too far distant from that recommended by computer models as the level required to potentially eradicate the disease.

The predicted outcome in the hypothetical scenario presented is conservative for three reasons. The true number of vaccinator nights during field trials was less than that reported, by an estimated ten percent. This is because minor operational problems meant that not all vaccinators were functioning properly at all times. It is also likely that some dyed possums were not trapped or correctly identified during the first four field trials, however, in the fifth trial an extremely intensive trapping program ensured that all possums on the study site were checked for dye.

Finally, the Jolly-Seber method used to estimate possum population includes a small proportion considered untrappable. This proportion would not be expected to use a vaccinator. Due to this the Jolly-Seber method slightly over estimates the possum population which may potentially be marked with dye, although not the population susceptible to tuberculosis.

In a field situation, the proportion of the total possum population exposed to the aerosol spray is reduced in several ways. For example, the density and location of vaccinators effects the proportion of the possum population encountering the device. Of the possums that encounter the device, not all will be attracted to it and of those that are attracted, not all will enter it. Finally, it is unlikely that every possum entering the device will receive an immunising dose of vaccine. The proportion of the population not effectively immunised will be dependent on the minimum immunising dose and the effectiveness with which vaccine is dispensed from the aerosol can. Further research is required to address the factors listed above that cause a proportion of investigating possums not to be vaccinated.

The influence of attractant on vaccinator efficiency

The attractiveness of a vaccinator to possums is one of the fundamental aspects influencing the success of the device. The use of an olfactory attractant greatly increased the intensity of investigation in pen trials and has been shown to increase the trap catch of wild possums (Cowan, 1987). However, considerable research, based primarily on olfactory attractants for possums, has failed to clearly identify any one substance as a superior lure (Todd, 1995, Morgan *et al.*, 1995).

Chocolate served as an effective attractant during the observation periods in the pen. In the field situation there was a rapid decline in attractive quality of the chocolate making it unsuitable for a long period of exposure. In particular, the quality was reduced by wet weather which allowed mould to grow. Attractant used in the bimonthly live trapping program was also effective when used in vaccinators. Apple segments dusted with cinnamon were more resistant to wet weather and mould. The powder coating also helped prevent dessication of the apple during hot weather. Although suitable, apple segments, acting as the vehicle for the cinnamon, were labour intensive to prepare and transport. Substituting the apple with a cube of foam rubber and cinnamon powder with cinnamon oil provided an improvement. Longevity of scent from cinnamon oil was similar to that of dusted apple.

Scented oil on foam is well suited to commercial production. Paste based products containing various scented oils are currently available for commercial possum control operations. However during vaccinator trials, quality of the attractant declined sharply after one week. It would be advantageous to extend the period during which the scent was acceptably strong. Volatility of the oil, which influences the speed of evaporation, may be manipulated during production to vary the strength of attractant and duration of dispersal. Automatic periodic release of a metered dose from an aerosol can would be ideal, but would probably be prohibited by the cost, due to complexity of the delivery mechanism. A similar solution may be the use of a porous cap on a container of cinnamon oil.

One alternative for incorporation of attractant would be to combine it with the vaccine in the aerosol can. A major advantage of this would be simplicity of use. The vaccinator operation would then only need to be checked to replace the vaccine or relocate the device. The functional life of the aerosol can would be the limiting factor. Unfortunately, it would be unlikely that sufficient scent would persist to attract possums if the vaccinator is not triggered periodically. During pen trials, the low degree of investigative behaviour shown toward novel objects without an attractant indicates that there would be little use made of a vaccinator in the wild without an attractant. Combining the attractant with other constituents of the aerosol can may also cause problems.

Effect of construction materials and appearance on vaccinator performance

The effect of construction material and shape of a vaccinator on attractiveness to possums was initially considered important. However, results of the first two trials and also pen trials indicated that wooden vaccinators were not attractive to wild possums. In addition, operation and adjustability of wooden vaccinators was also inferior to the steel models due to the mechanical qualities of wood. The wooden vaccinators developed mechanical problems when they became swollen in wet weather, causing stiffness of moving parts and rendering the device inoperable. Contraction due to drying lead to the development of a small amount of movement in critical areas causing the triggering mechanism to discharge erratically. At the conclusion of the second field trial the operation of the wooden vaccinators had deteriorated to the point where this model was abandoned.

The reliability of a steel vaccinator in the field trails was excellent. The device was generally very robust, however two modifications were required. Firstly, loss of the nozzle from several aerosol cans meant that improved protection of the triggering mechanism was necessary. The second modification was a small change in trigger spring tension, as a slight but constant trigger pressure caused several cans to leak until empty. Vaccinators would also benefit from a partial or complete floor to stop vegetation growing into the moving parts of the device.

In the event of aerosol vaccination being accepted as a useful method for the control of tuberculosis, copies of the vaccinator would be required in large numbers. The most likely method of production at present is an injection moulded plastic frame and pressure plate and steel parts for the triggering mechanism. Injection moulding is currently used to produce a range of trap designs, including the ‘Timms Trap’, for possum control. The advantages of injection moulding are low cost and qualities of the construction material, including strength, resilience, longevity and light weight. Two important considerations when designing a plastic mould include the rigidity of certain key areas, particularly the pivot shaft for the trigger and aerosol can retainer to minimise flex. The second consideration is ensuring key parameters of the plastic mould match the steel prototype exactly to minimise costs, as no adjustment would be provided in commercial models.

Factors effecting density of vaccinator distribution

The optimum density for vaccinator distribution will depend on a range of factors including attractant strength, possum density and physical environment. Definition of a target proportion of a possum population to be protected would be the first step. Computer models of tuberculosis in possums can provide this information (Roberts, 1996), though the limitations of such models must be taken into account. The second step would be a field trial using dye to determine if the proportion of wild possums using the device is sufficient to meet the requirements of the computer model. The third step would be to measure the protective effect of vaccine when dispensed from the vaccinator. Vaccinator field trials conducted to date have been exploratory and results, although encouraging, would benefit from the support of additional research.

The use of bait stations did not result in a large increase in the number of dyed possums, given a fixed vaccinator density. Their function was to provide a food reward for possums visiting the site, but they were not used as pre-feeders. Faecal evidence indicates use of bait stations was primarily by possums but also by rats. It is possible that possums were feeding at a bait station though not entering the vaccinator nearby. Studies have shown bait stations to attract wild possums and increase kill rates by poisoning when used as pre-feeding stations (Morgan *et al.*, 1995, Hickling and Thomas, 1990). A guide to an optimal density of vaccinators is provided by research on bait station use. Thomas and Fitzgerald (1995) reports that 90–100% of possums would feed at these points over a two week period when they were spaced at 50 m intervals. This figure fell to 60–70% when placed at 500 m intervals. These results may not be strictly applicable to vaccinators as the possums received a food reward at a bait station, which would not be the case with a vaccinator. This may be overcome if a bait station was included at each vaccinator site.

Vaccinator density must take into account the ranging characteristics of possums. Jolly (1976) found home ranges varied from 0.32 ha to 3.64 ha with male ranges larger than females. These estimates are representative of studies summarised by Cowan and Clout (2000). Adult possums establish home ranges from which they do not generally make permanent movements away from. Possums use some parts of their habitat and home range much more frequently than others (Leyhausen, 1965). For example favoured denning areas, food sources and the bush pasture margin. Identification and placement of vaccinators in the areas of frequent use would greatly increase the proportion of the population exposed to the device.

Tuberculous possums are clustered in space and time, in ‘hotspots’(Coleman, 1988). Vaccinator placement in hotspots would protect possums with the highest risk of exposure to disease. Hotspots can be identified using the local knowledge of commercial trappers and farmers in conjunction with information provided by hotspot predictor software such as the product EpiMAN (TB) (McKenzie and Morris, 1995).

Is a vaccinator attractive to animals other than possums?

No risk of physical harm to non-target species was observed during pen or field trials. Several species in addition to possums were seen to investigate a vaccinator when in the pen. Mice and rats frequently searched for the attractant, although they were too light to trigger the device. In the field, during the depopulation of the study site, eleven adult hedgehogs were trapped. One of these had been marked extensively with dye. The weight of a hedgehog is approximately half that of a possum and it is possible other non-dyed hedgehogs had entered the device and crossed the pressure plate without activating the spray. The attractant was well protected and the main problem resulting from inspection by hedgehogs would be that the animal may become trapped in the second chamber of the vaccinator. However, there was no evidence of this occurring during the trials.

There was no evidence of disturbance of vaccinators by wild pigs, deer and goats during the trials. One vaccinator was found tipped over though this was probably caused by the wind as it was in an exposed position and not secured to the ground. The only damage to a vaccinator was caused when it was trodden on several times by cattle, destroying the device completely.

Further research is recommended to determine behaviour of native flightless birds toward the vaccinator. However, observations indicate that investigation of a vaccinator is unlikely to represent a risk of injury to any species found in New Zealand.

What is the next step?

The concept of a self-setting aerosol dispenser has been proven in a basic form. There are now three goals to be accomplished before vaccination of possums can be considered for large scale use in the control of tuberculosis. The first is to develop the BCG vaccine to be dispensed from an aerosol can. Vaccine research should be directed at finding a liquid vehicle suitable to maintain a live microorganism with minimal loss of viability. The vehicle in which the vaccine is suspended must prevent settling out or clumping of the microorganism. The combination of vaccine and mechanical components to generate aerosols as close to 5 ?m in diameter must also be determined. This combination will be dictated, to some extent, by the physical and chemical properties of the vaccine and vehicle. Survival of BCG vaccine in an aerosolised form must also be understood (Heidelberg *et al.*, 1997). Similar research goals to those listed above have already been achieved, for example in the use of aerosolised bacteria (*Streptococcus suis*) to vaccinate pigs (Brown *et al.*, 1997) and the culture of a range of micro-organisms after aerosolisation (Cox and Wathes, 1995). Following completion of the development of the vaccine formulation, the vaccinator must be modified to dispense the aerosol to the eyes and nose of a possum. In addition a comparison of spray and mist type discharges of aerosol may help determine the most effective method of vaccine penetration.

The second will be to determine what proportion of a possum population will use a vaccinator. This knowledge will provide a much more accurate prediction of the capabilities of the device. It would also be beneficial to identify the major influences on success rate, for example, the effect of attractant type, vaccinator density and the use of bait stations.

The third goal is to measure the efficacy of the vaccine in possums when dispensed from a vaccinator. If it becomes apparent that this rate is low, further research will be required to identify and correct whichever part of the vaccine, or vaccinator, is responsible. If the concept remains sound after these three goals have been achieved, the commercial production and distribution of the device and aerosol cans should be investigated.

There is potential to use an aerosol delivery device for purposes other than vaccination. Any substance that can be effectively dispensed as an aerosol, (by the intra-nasal or, possibly the conjunctival route) may be administered to possums.

Large scale poison based possum control operations are the source of increasing public concern regarding animal welfare and environmental issues. Biological control of possums in New Zealand is a possible alternative to poisoning. Research in microbiology and genetic manipulation specific to possums is bringing this possibility closer to reality. Antigens engineered to target various physiological aspects such as fertilisation, immunity or lactation (Cowan, 2000) could be distributed by a range of possible biological vectors, in particular poxviruses, adenoviruses, herpesviruses (Smith, 1996) and BCG. An aerosol delivery device would be well suited to the distribution of these viral or bacterial vectors containing antigen.

Biological control of possums may also be possible by the distribution of a disease causing agent by an aerosol delivery system, such as the virus causing wobbly possum disease. Urine, blood, tissue suspensions and homogenised mites have all been proven infectious, and present a range of options for the formulation of constituents within the aerosol can. Further transmission is by close contact between possums (Perrott *et al.*, 2000).

There is potential to modify the vaccinator design to suit species other than possums. Badger culling operations in Ireland and Great Britain have reduced the prevalence of tuberculosis in cattle but have failed to eradicate the disease. Prevalence of disease ranges from zero to approximately eight percent (Cheeseman *et al.*, 1989), similar to that in possums. Unlike the possum, which is a serious pest, badgers are a protected species in both countries and alternative methods of disease control are required as public disapproval of badger culling increases (Clifton-Hadley, 1996). Badger populations are smaller and more accessible than the New Zealand possum population. Vaccination of badgers with BCG is a possible control method. Research has demonstrated enhanced cell mediated immunity, delayed excretion of the organism and prolonged survival of infected badgers following vaccination (Stuart *et al.*, 1988). Currently there is no delivery mechanism for vaccine to this species, however the aerosol vaccinator could be modified to suit delivery of BCG to badgers. Adaptation would require an understanding of both the physical and behavioural aspects relevant to the vaccination of badgers, or any target species.

3.10. CONCLUSION

The initial concept of an aerosol vaccinator has been transformed into a functional device which has been used to apply aerosolised dye to wild possums in a series of trials. Neophobia and social structure were two key aspects of possum behaviour evaluated with respect to the use of a vaccinator. Most possums were found to exhibit minimal neophobia and would actively investigate novel objects. A loose social structure was present in captive possum populations with one dominant animal and a changeable hierarchy in the remainder of the group. Social structure influenced the order in which possums would investigate a novel object, but did not restrict investigation to any subset of individuals within the group.

A simple, robust vaccinator was designed that met all five of the essential design criteria. The design path of the vaccinator covered fourteen different stages. In its final form the vaccinator is attractive to possums and will discharge an aerosol can as the possum approaches an attractant.

A series of exploratory field trials provided preliminary results on the use of vaccinators in a wild possum population. Over eight months and 289 vaccinator nights, vaccinators marked 56% of the study population. Computer modelling of tuberculosis in possums suggests that protection of a threshold proportion of the population reduces disease prevalence in the total population and may lead to its eradication. The proportion of the possum population marked by a vaccinator during the field trials was less than the threshold reported in one model but greater than that given by a second model.

The current results are encouraging, and justify further research in two directions. Firstly, development of a vehicle to maintain the viability of live BCG vaccine in an aerosol can. Secondly, further testing is required to determine the most effective density of vaccinators and method of distribution, and to study other factors influencing the proportion of a possum population that will use the device.

If additional research continues to provide encouraging results, the next step would be replication of the vaccinator in large numbers. Aerosol vaccination with BCG would then be ready for use in conjunction with existing control measures as an alternative method of controlling tuberculosis in wild life possums.

Regardless of the success of aerosol vaccination in New Zealand, the concept may be suitable for distribution of other aerosolised material to possums. It may also be suitable for use in dispensing BCG to badgers which act as a wildlife reservoir of tuberculosis in Great Britain and Ireland.

Chapter 4

GENERAL DISCUSSION

In the first study, the denning and ranging behaviour of possums infected with tuberculosis was observed from clinical stages of disease until death. Anecdotal reports had suggested terminally ill possums relocate from their established denning and activity range to remain on or near pasture, which is a preferred food source. This behaviour would greatly increase the chance of tuberculous possums interacting with, and transmitting disease to livestock.

The den sites of both tuberculous and healthy possums were predominantly clustered on approximately one quarter of the study site. The denning range of individual possums was most frequently (10/22 possums) a single cluster of dens or two distinct but adjoining clusters (8/22). Four possums used either three distinct clusters of dens, or dens which showed no clustering. The activity range size was larger than most home range reports (Cowan and Clout, 2000) and showed substantial variation between individual possums. Half of tuberculous and half of healthy possums made infrequent long range (>200 m) movements from their normal activity range with a median length of 273 m (range 210–670 m).

Naturally tuberculous possums survived for a median of 11 weeks (range 1–84) from identification of clinical disease. During most of this period their behaviour was indistinguishable from that of healthy possums. However, there was a rapid decline in the physical condition of the possum during the terminal stages of disease which lasted approximately 2–3 weeks. This was characterised by lethargy, weakness, disorientation and wandering during daylight. Most tuberculous possums (15/17) died in scrub or in areas of long grass associated with scrub. These cases were a potential source of infection to wild deer, ferrets and pigs. A small proportion (2/17) were found on pasture.

Hotspots are denning areas where tuberculous possums are found in unusually high density. This study identified one major and several minor hotspots. The location of the major hotspot varied by 200m depending on whether it was defined using trapping or denning information. The presence and location of some hotspots was found to change with time, while others remained stable across an eight year period (Pfeiffer, 1994).

This was the first study to specifically target the behaviour of tuberculous wild possums and evaluate the influence of this behaviour on the risk of disease transmission to livestock and other possums. The study would be best improved by the generation of more data, ideally with a greater number of possums. Unfortunately, in practice data collection was limited by the number of clinically tuberculous possums which were trapped. Alternatively, the frequency of tracking could be increased. Data quality could be improved by intensifying the tracking of possums during the terminal stages of disease. To increase the volume of data and more accurately record the movements of terminally ill possums a radio collar could be developed which periodically stores GPS information, enabling possum movement to be mapped. Use of this technology would facilitate the accurate recording of long distance movement, and the proportion of time a terminally ill possum spends in scrub or pasture habitat.

The spread of tuberculosis is largely dependent on host density (Zuckerman, 1980). Possum control in New Zealand is focussed on the reduction of host density below a defined threshold, which breaks the transmission cycle. This level is determined by computer modelling to be 43% of the habitat carrying capacity by (Roberts, 1996). Initial control is generally achieved by poison bait distribution by aircraft, reducing the possum population to approximately five percent of pre control numbers. Annual or bi-annual ground control operations maintain this low possum population. The main technique used to measure possum abundance is based on residual trap catch when traps and attractant are set to a standard protocol for three consecutive nights. Two other methods of monitoring possum abundance are faecal pellet counts and measuring bait taken from bait stations. The success of control measures is evaluated by the levels of tuberculosis in cattle herds within the controlled area and the prevalence of infected possums during trapping operations (Warburton, 2000). Current techniques have substantially reduced the percentage of infected cattle herds to 0.7%, but have not yet reached the international standard of 0.2% (Coleman and Livingstone, 2000). However, despite these control measures and the investment of approximately NZ$50m annually, tuberculosis has continued to spread, with infected possums found over one third of New Zealand in 2000 (Animal Health Board Annual Report, 2000).

It appears that aerosol transmission is the most likely route of infection between tuberculous possums and livestock (Paterson and Morris, 1995). As healthy possums actively avoid cattle it is suggested that the majority of transmission from wildlife to livestock occurs from debilitated terminally ill possums. If this is true, there are three issues which make eradication of these high risk possums and subsequent reductions in the number of infected herds extremely difficult. Firstly the prevalence of tuberculous possums is usually low (1–15%) with only a proportion of this group terminally ill at any one point in time. Secondly, it is not possible to predict the period for which a possum will carry the disease before entering the terminal stage. The duration of survival of tuberculous possums is effected by environmental stress such as wet and cold weather conditions and physiological stresses associated with nutrition and lactation (Pfeiffer, 1994). As a result of this, deaths generally show a seasonal pattern, though are difficult to predict with any greater accuracy. Thirdly, possum behaviour during the terminal stages of disease may be erratic due to the loss of awareness and debilitation. It can also include long range movements outside the established range of the diseased possum, though not necessarily toward pasture. In short, temporal patterns of abundance of terminally ill possums cannot be predicted accurately. In addition they comprise a very small proportion of the total possum population and may behave in an erratic manner. As a result of this terminally ill possums are an extremely difficult group to target during standard control operations.

To reduce the spread of tuberculosis it is important to more effectively cull tuberculous possums, with the intention that they are removed before entering the terminal stages of the disease. This can be achieved by more intensive identification and targeting of permanent hotspots and more frequent poisoning in these areas. The use of local knowledge, primarily from land owners is very important in the detection of these areas, particularly as the results of this study have shown hotspot location may change over several years. This study suggests temporary hotspots may be the result of movements of tuberculous possums during which they are trapped. On the basis of this, they are not considered important, with emphasis placed on targeting the source of diseased possums (permanent hotspot) rather than the areas they may possibly move to.

Targeting tuberculous possums with greater accuracy will improve the efficiency of control operations and reduce the cost of disease control on a per possum basis. This is particularly important as the readily achievable reductions in the number of infected herds, as a result of possum control, are already in effect. The cost of eradicating a progressively higher proportion of the wildlife reservoir of tuberculosis rises very quickly and in the extreme will almost certainly be unacceptably high. There is currently no indication that all possums or tuberculosis will be eradicated from New Zealand.

The challenge of controlling a wildlife reservoir of tuberculosis is not unique to New Zealand with similar situations existing in several countries. Examples include the badger (*Meles meles*) in England and Ireland, the Australian swamp buffalo (*Bubalus bubalis*), the white tailed deer (*Odocoileus virginianus*) in America and a number of species in Africa. Only in Australia has the disease been brought under full control (Cousins *et al.*, 1998). For almost twenty years an extensive test and slaughter scheme required the mustering of both wild and domestic cattle and buffalo over extremely large areas. It also required the detection and destruction of those animals which could not be collected for testing. However small scale breakdowns continue to occur, one of which has involved at least 20 properties (Kopecny and ABC National Rural News, 2000). Control strategies which were successful when applied to the Australian situation, are not well suited to New Zealand due to the range and accessibility of wild species involved. An alternative solution is required to control or eradicate tuberculosis from New Zealand.

The wild fox (*Vulpes vulpes*) functions as a wildlife reservoir for rabies in Europe. The epidemiology of rabies in Europe is similar to that of tuberculosis in possums in New Zealand. It generally moves in cycles, drops to very low levels when host levels are low and has a prevalence of 3–7% in areas where rabies is endemic (Anderson *et al.*, 1981). Vaccination of foxes by oral bait distributed twice annually has proven a more effective method of disease control than the traditional methods based on the shooting and gassing of fox populations. Over a ten year period the incidence of rabies in France has decreased by 99% and eradication of the disease from this country now depends on control in surrounding countries. In some other areas in Europe rabies has been completely eliminated and vaccination is no longer required (Aubert, 1996).

The vaccination of wild possum populations against tuberculosis is one alternative to the current destruction-based control techniques. Administering BCG vaccine to captive possums by a variety of routes has resulted in varying levels of protection. Aerosol and subcutaneous methods have resulted in the highest level of protection in trials by Aldwell *et al.*, (1995). Successful control of tuberculosis would not require vaccination of the entire possum population. Instead when the protected proportion of a population reaches a threshold level estimated at 54% by Barlow (pers. com., 2000) there are too few susceptible individuals in the population for the disease to persist. This is the concept of herd immunity.

The use of vaccination as a means of controlling tuberculosis in wild possum populations provides the opportunity to control or even eradicate tuberculosis in New Zealand without having to achieve the more difficult task of eradicating the possum completely.

One of the fundamental aspects of the concept of vaccination which remains to be evaluated is the method of vaccine distribution. Currently, the two most likely possibilities for delivery are by aerosol, or by oral bait. When considering an aerosol delivery mechanism there are five design criteria which must be met. These are that the device is attractive to and easily used by possums, that it discharges an aerosol can in some way, that it is of simple design and robust.

Pen trials were used to develop a device which would meet the design criteria. They were also used to evaluate the effect of neophobia and social hierarchy on the way in which possums used the device. It was found that most possums would actively and thoroughly investigate a range of novel objects including various vaccinator designs. Social hierarchy was found to have some influence on the order in which possums would approach an object, though a small proportion of possums did not approach any novel object.

A series of field trials were used to test the capacity of the final vaccinator design to attract possums in the wild and apply marker dye to the back of the head and shoulders. These trials were conducted over eight months and at their conclusion, 56% (46/82) of the adult possums had been marked with dye.

The pen trial results would have been improved by using mixed sex possum groups to identify the effects of sex on social hierarchy. The collection of data during field trials could have been improved in several ways. For example, the use of time lapse or movement activated photography would have provided interesting information on the use of individual vaccinators including investigation or use by other species. Alternatively, the incorporation of a counter mechanism to record the frequency of vaccinator activation would have produced some of this data. The use of different coloured dyes would indicate if possums were travelling between vaccinator sites.

The encouraging results from the field trials combined with existing literature on the vaccination of possums with BCG provide strong evidence for continued research into the possibility of vaccinating wild possums with an aerosol delivery device. At this stage there are three key avenues of research which need to be followed.

Firstly, due to the time constraints of this study, field trial results were preliminary estimates of vaccinator use by possums. A more accurate estimate of the proportion of a wild possum population activating a vaccinator during a set period is required. Following this the optimum, or most efficient period of vaccinator deployment can be determined. It is possible to collect this data with the current vaccinator design and data quality would be improved by incorporation of the points mentioned above. The effect of manipulation of vaccinator density on the number of dyed possums should also be investigated.

The second avenue of research relates to ensuring other species do not use the vaccinator. The species most likely to be a problem are those which are attracted to substances used to attractant possums and in particular those which are similar in weight and size to a possum. Some native flightless birds may fit into this category.

Finally a container for the aerosolised BCG vaccine must be formulated. This will probably be the most time consuming and technically involved component of vaccinator research. As the BCG vaccine is a living organism, it may not be possible to use conventional propellants and vehicles to dispense the aerosols. The longevity of the organism in the environment must also be investigated. The tilt action valve is available in many aerosolised products and effective at this stage, but investigation of the other nozzle types may be required to ensure appropriately sized aerosols are generated and in a suitable distribution pattern. With the container finalised the vaccinating device can then be modified to dispense vaccine to the nose and eyes of investigating possums. At this point the first trials using aerosolised BCG vaccine to protect wild possum populations against tuberculosis can commence.

On the assumption that the results of suggested future research remained promising, the following step would be production of vaccinators and vaccine in large numbers. Production of the device would probably be based around an injection moulded plastic frame and pressure plate combined with the finer parts made from stainless steel. This would ensure the devices are light, very robust and long lasting. There are existing commercial facilities for the production of attractant to be used in the vaccinator and the production of aerosol cans.

A logistical frame work made up of the regional councils and contract hunters currently used to conduct ground based maintenance control by poisons could be quickly adapted to the dispensing and servicing of vaccinators. They could also be provided for sale to enable land owners to initiate their own vaccination programs.

Vaccinators could be used in conjunction with established possum control methods to establish and then gradually enlarge areas of disease free possums. This would be achieved by the use of vaccinators within buffer zones and areas previously subjected to possum control (Fraser *et al.*, 1998). The vaccinator and buffer zone concept would initially be used over small areas, for example a group of farms with a persistent tuberculosis problem. The first step is reduction of the existing possum population by 70–90%, which is standard control procedure. Vaccinators could then be distributed within the controlled area and buffer zone. It is suggested that the combination of possums protected against tuberculosis and reduced population density would satisfy the requirements of herd immunity. The result is a disease free possum population. During the initial control operation a buffer zone three kilometres wide (Fraser *et al.*, 1998) would be created to stop the outlying possum population recolonising the controlled area. Maintenance control is used to keep the buffer zone below 40% of the pre control population as recommended by (Nugent *et al.*, 1997). Vaccinators must be redeployed periodically. The duration between settings remains to be defined and is based on the duration of vaccine protection and the most efficient period for targeting possums that have recently moved into the control area. A conservative estimate of every six months is presented here. The control of possums outside this protected area and movement of the buffer zone into this newly controlled area provides a method of extending the disease free possum population. In short, this technique theoretically provides a tuberculosis free possum population, beginning with the areas in which the problem is most severe.

In Great Britain and Ireland the badger is the wildlife reservoir of tuberculosis associated with the persistence of this disease in livestock in these countries. This situation has many similarities with the possum in New Zealand. Badgers are normally nocturnal and share pasture with livestock while hunting earthworms, though actively avoid the approaches of the livestock (Benham, 1985). They live in colonies in under ground ‘setts’ though forage as individuals. Badgers infected with tuberculosis exhibit behavioural changes including debilitation, wandering during daylight, sometimes in or around farm buildings and do not display natural avoidance behaviour of other animals including humans (Cheeseman and Mallinson, 1981). Badgers excrete the organism in greatest numbers in urine when they have renal lesions and also in sputum, pus and faeces. The actual mode of transmission from badgers to cattle is unclear, though cattle have been shown to strongly avoid pasture contaminated with badger urine or faeces, indicating it is by some mode other than pasture contamination (Benham, 1985). The close confines of a sett provide excellent conditions for the transmission of infectious aerosols between badgers.

The first tuberculous badger was identified in Great Britain in 1971 and during the following decade large numbers of badgers were killed, mainly by gassing, as part of the disease control program. In 1973 badgers became officially protected, though government agencies were given licence to continue with control programs. These control operations greatly reduced the number of infected herds, though failed to eradicate the disease. There are now strongly polarized public views on the issue of continued badger culling. These views are a result of failure of control operations to eradicate the disease, ongoing financial investment and media attention. Given these factors and constraints it appears logical that the vaccinator concept could be modified to suit use by badgers. This would provide a very timely opportunity to control tuberculosis while preserving badger populations in Great Britain and Ireland.

If the vaccinator is successful in reaching a large proportion of wild possums, there is potential to explore other forms of aerosol therapy, even if the concept of vaccination with BCG is unsuccessful. One avenue currently under research is that of fertility control. The main targets for this form of possum control include interference with fertilisation, embryonic development and lactation. This would be achieved through the genetic modification of naturally occurring properties of possum physiology. The vector by which this technology is delivered would probably be of viral or bacterial type and could possibly be dispensed as an aerosol. This would remove the potential problems associated with introducing additional parasites or diseases of the targeted pest to carry out this task. It would also provide a more accurate measure of the proportion of the target population which were exposed (Montague, 2000).

In conclusion, attempts to control tuberculosis in livestock and wildlife, in particular the possum have now been in operation for approximately 30 years in New Zealand at a substantial and ongoing financial cost. In more recent years the persistence of this disease in both domestic and wild animals has been largely attributed to infected wild possum populations. Although current methods have been an effective means of reducing the number of infected herds they have not managed to meet the international requirement of 0.2%, representing freedom from disease. Current control operations have also failed to stop the geographic spread of the disease in wildlife. The heavy reliance on poisons for possum control is the source of increasing public and international concern with respect to animal welfare and the effect of large and repeated applications of poison on the environment.

The concept of vaccination of the possum provides what appears to be an attractive alternative to the control measures currently in use. This concept targets exactly the same goals as current control strategies and could easily be incorporated into existing control techniques. If the concept proves successful, there is the opportunity to modify the delivery mechanism to suit other species. There is also the potential to distribute other aerosolized materials to wild animal populations.

Appendices

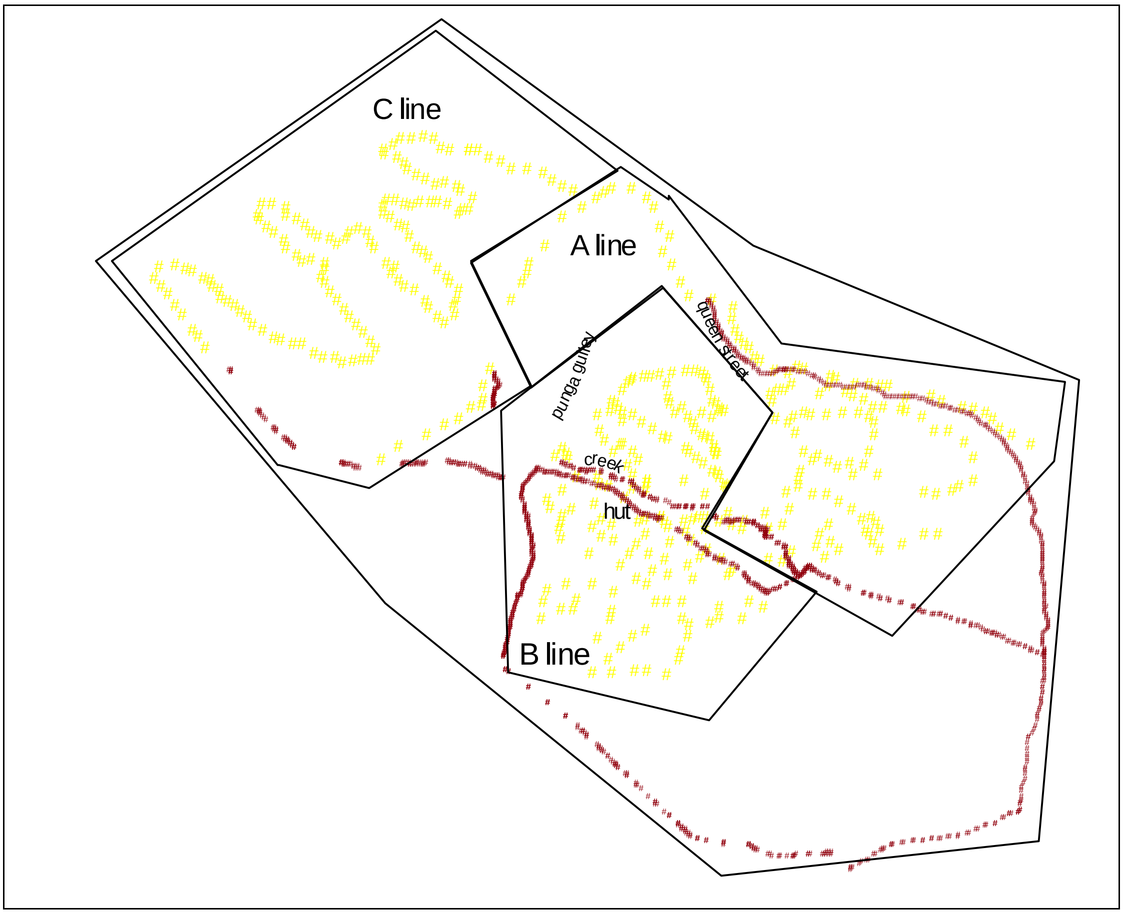
APPENDIX I

Trap sites on the Castlepoint study site.

Where present, contours represent density of traps which caught the respective possum groups, from high (central most 20% of used trap sites, darkest shade), through 40%, 60%, to low (80% of used trap sites, lightest shade).

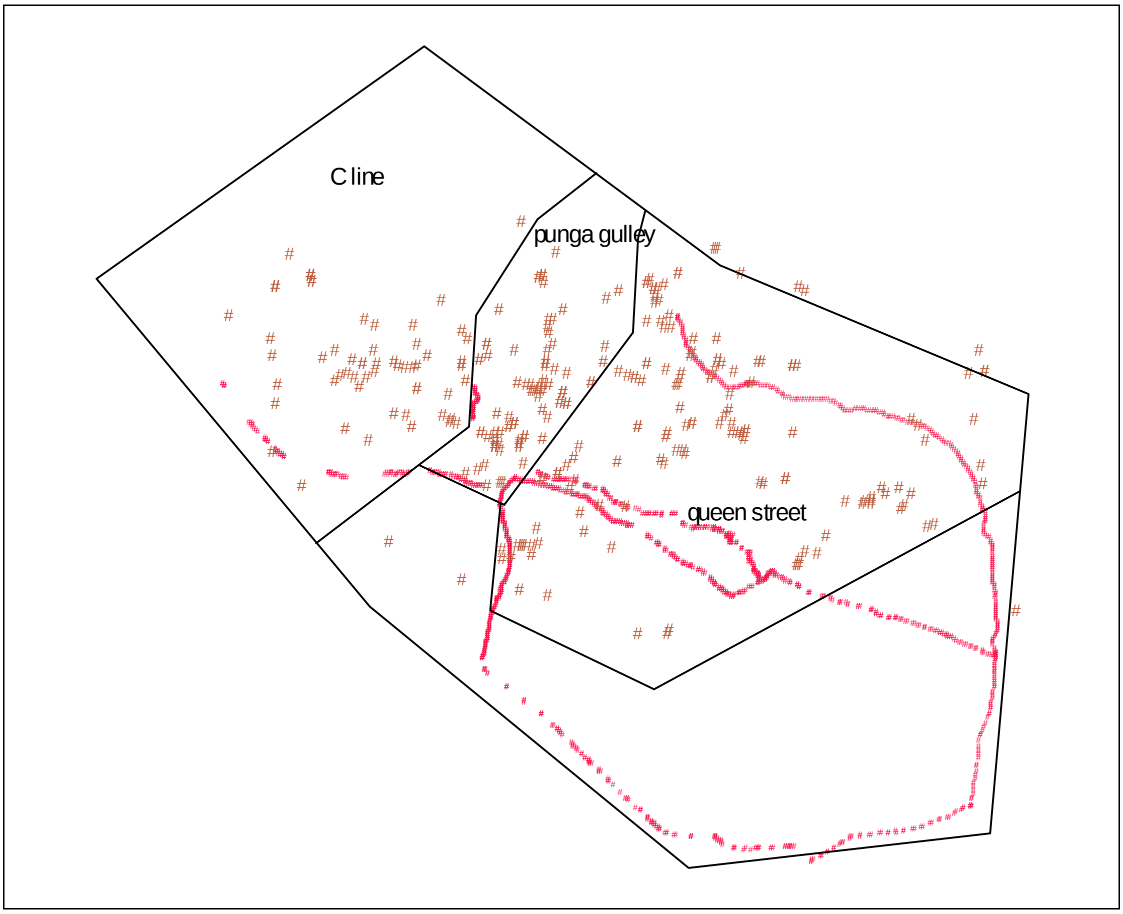
Appendix 1.1

Distribution of trap sites on the 40 hectare Castlepoint study site. Scale: 1:11000
Dots represent traps, the thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks.



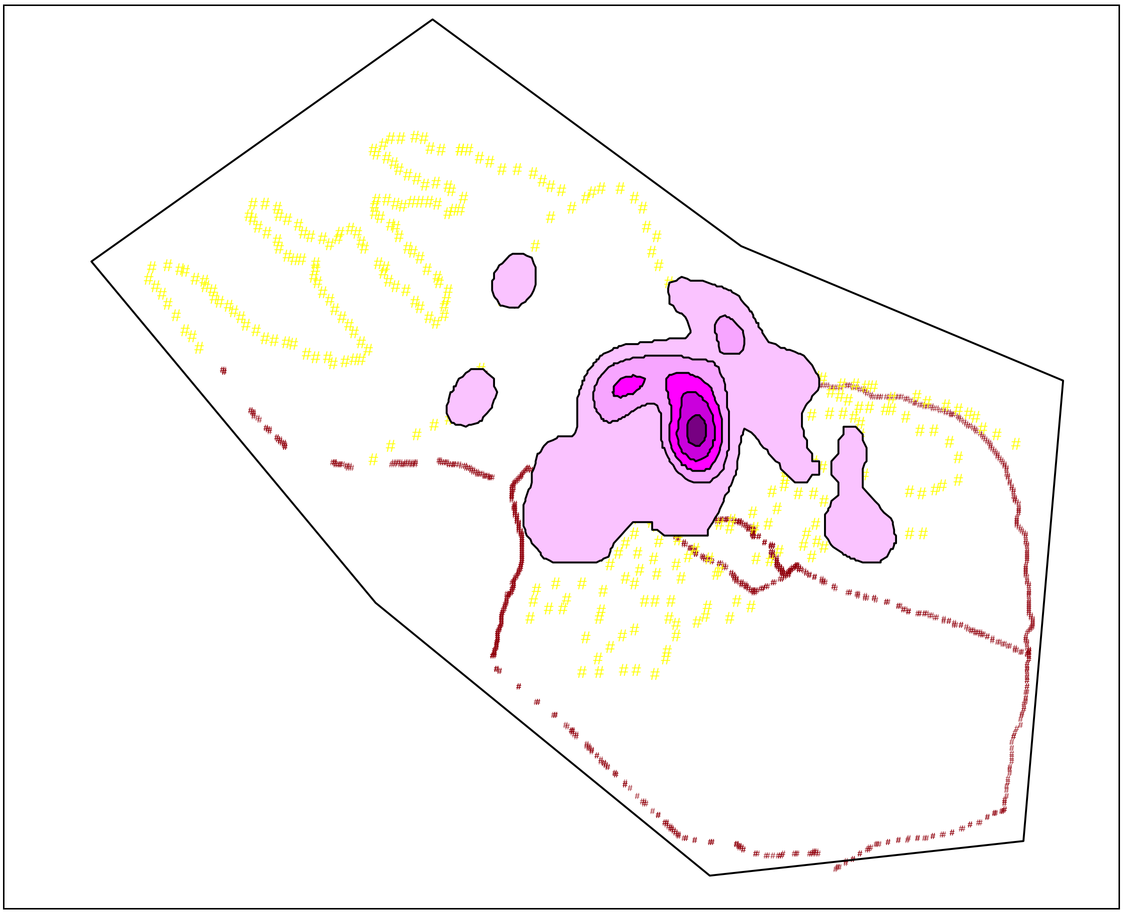
Appendix 1.2

Study site divided into the three areas of different den site density. Punga Gulley represents a high density of den sites, Queen Street represents medium density and C line represents low den site density. Scale 1:11000



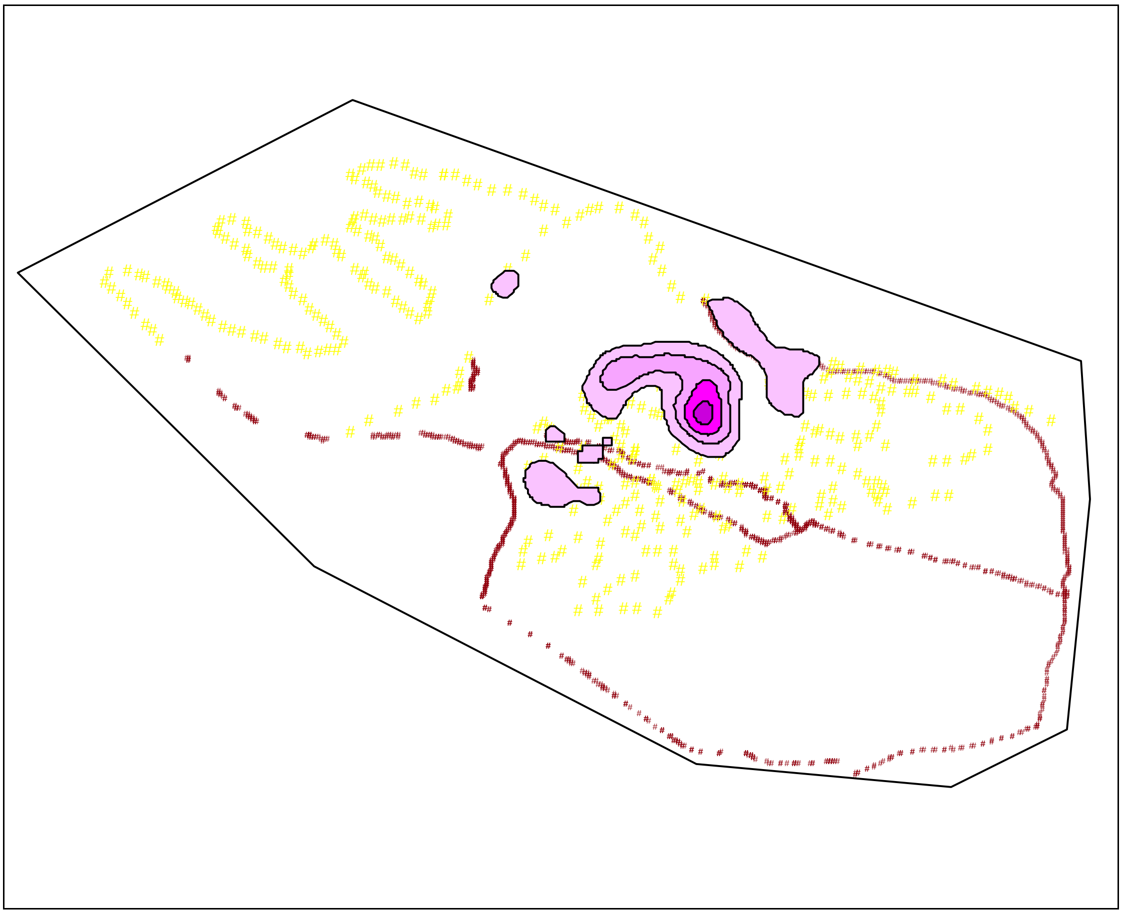
Appendix 1.3

The kernel density estimate indicates where all radio collared possums were most frequently trapped. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000



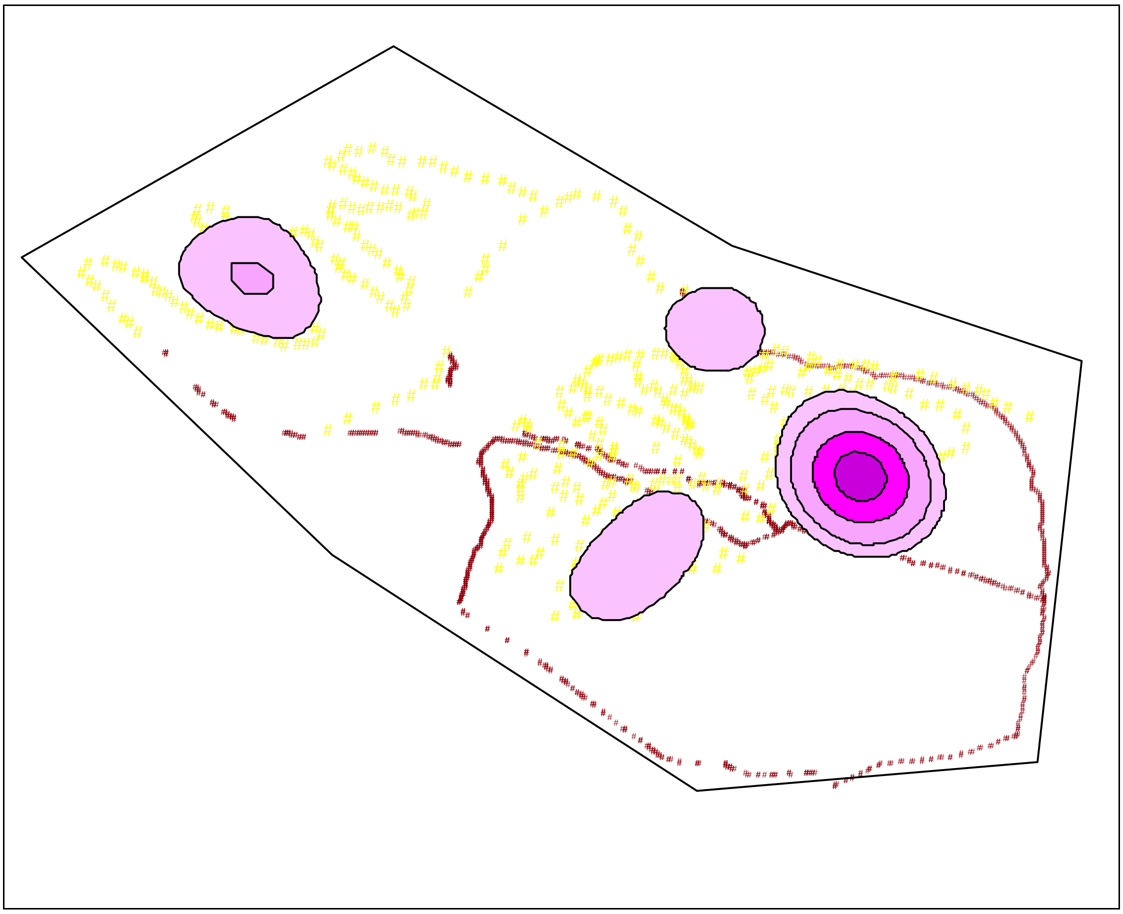
Appendix 1.4

The kernel density estimate indicates where radio collared tuberculous possums were most frequently trapped. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000



Appendix 1.5

The kernel density estimate indicates where radio collared healthy possums were most frequently trapped. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000

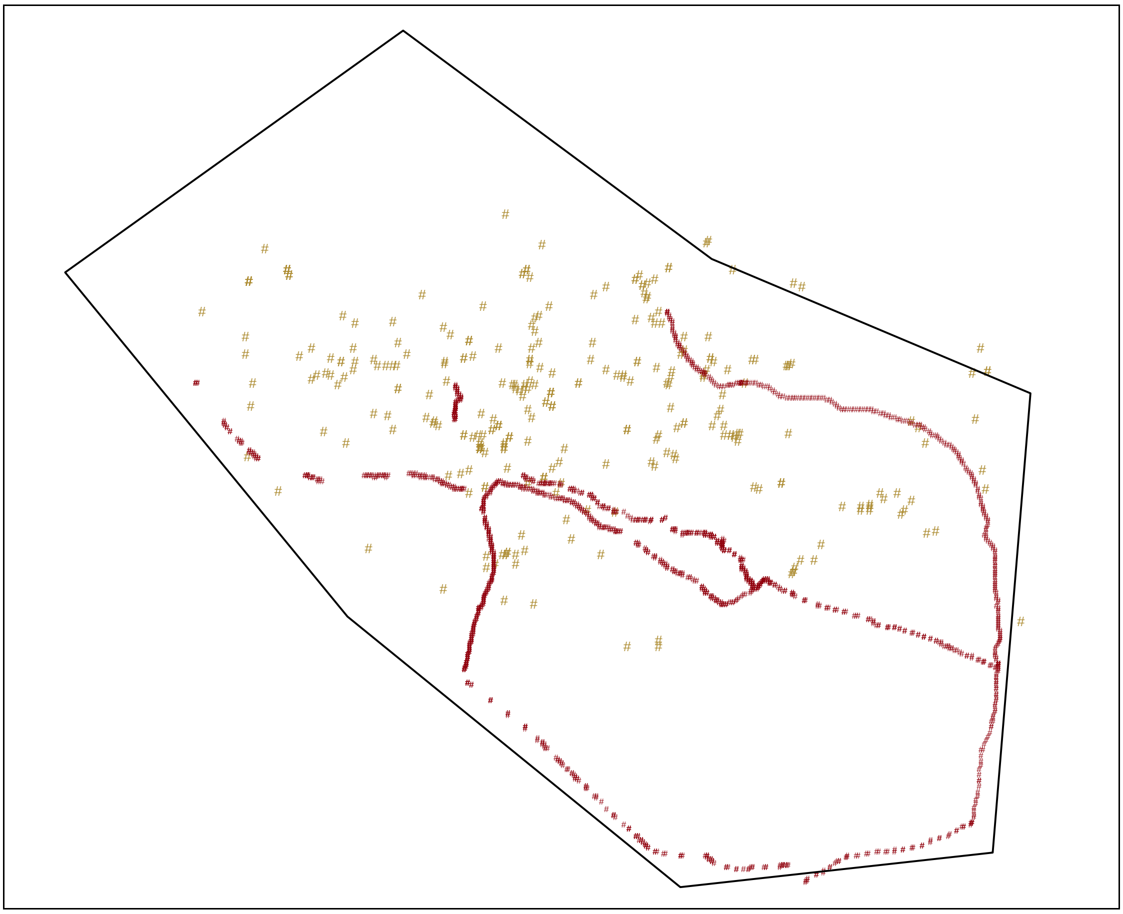


Den sites on the Castlepoint study site

Where present, contours represent den site density for respective possum groups, from high (central most 20% of den sites, darkest shade), through 40%, 60%, to low (80% of den sites, lightest shade).

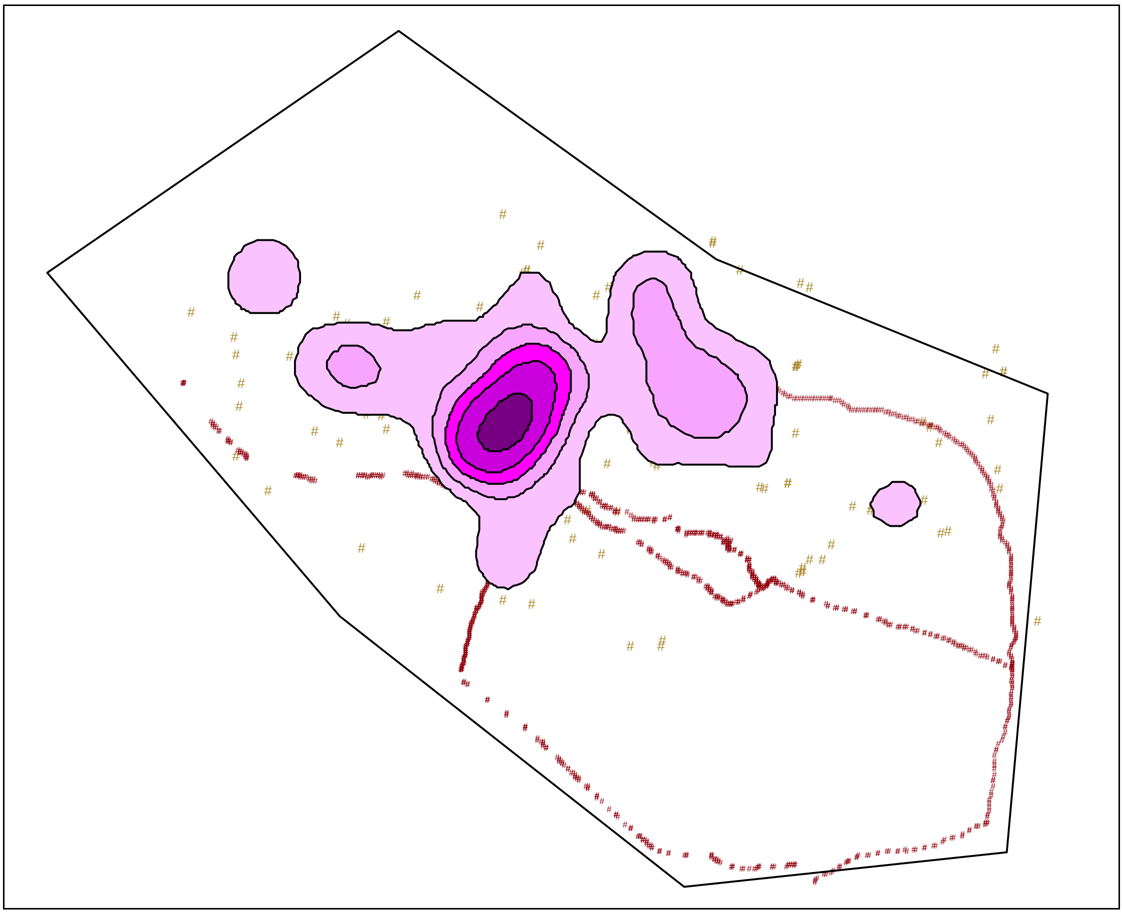
Appendix 1.6

Distribution of den sites for all radio tracked possums on the study. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000



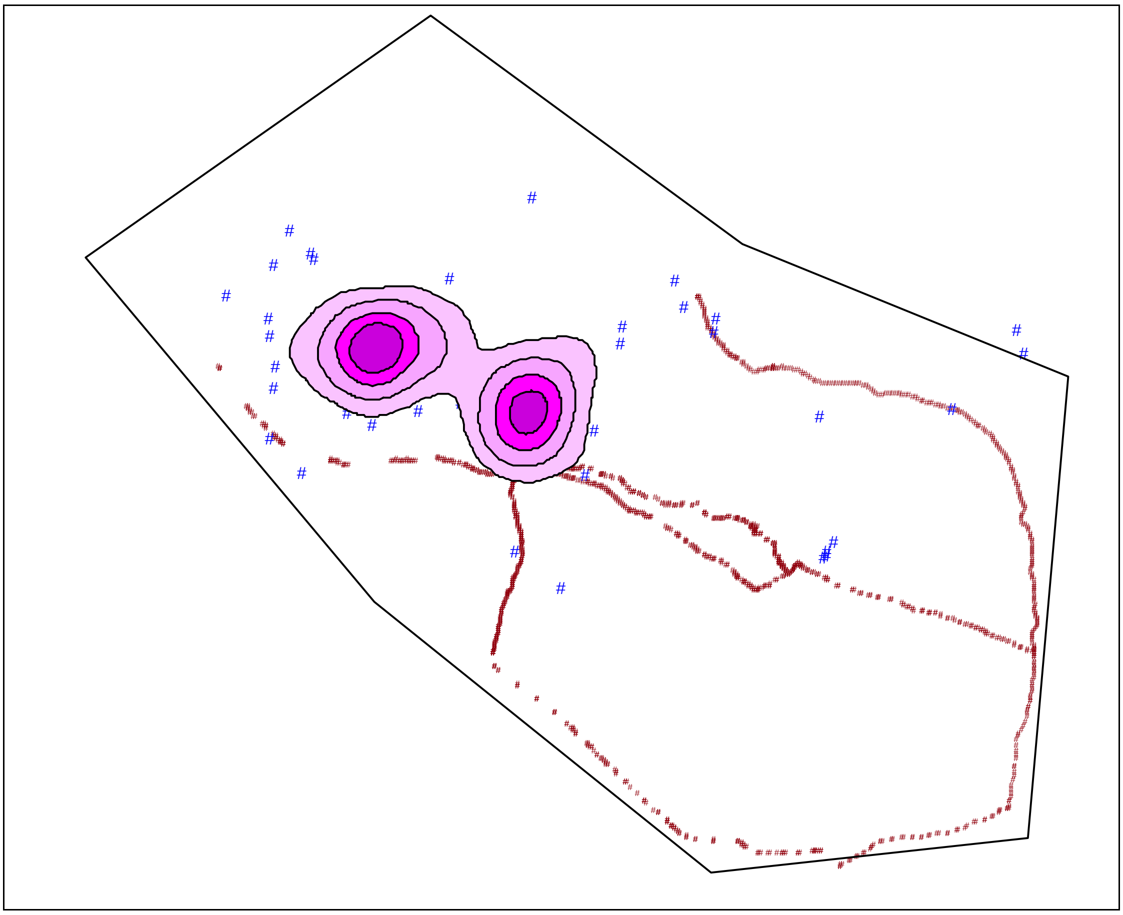
Appendix 1.7

Kernel density estimate of den sites for all radio tracked possums on the study site. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000



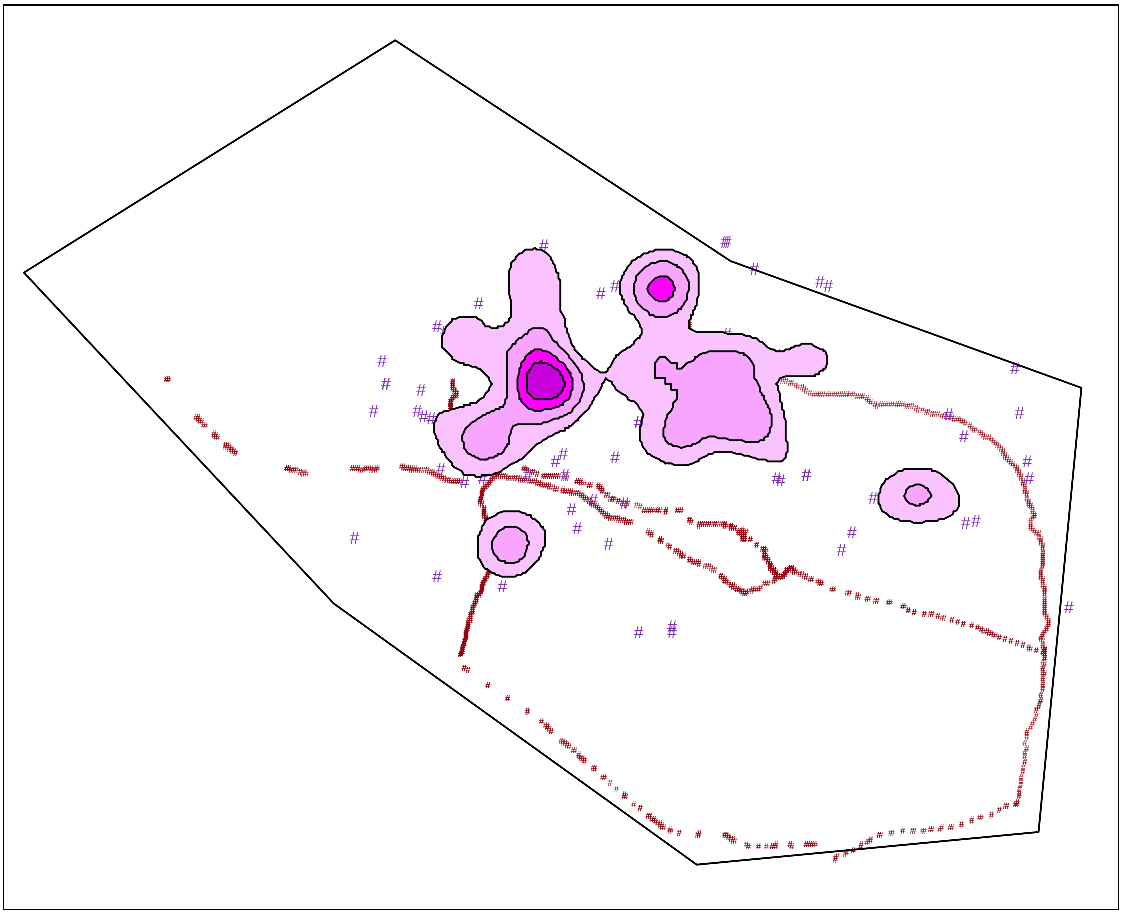
Appendix 1.8

Kernel density estimate of den sites for radio tracked healthy possums on the study site. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000



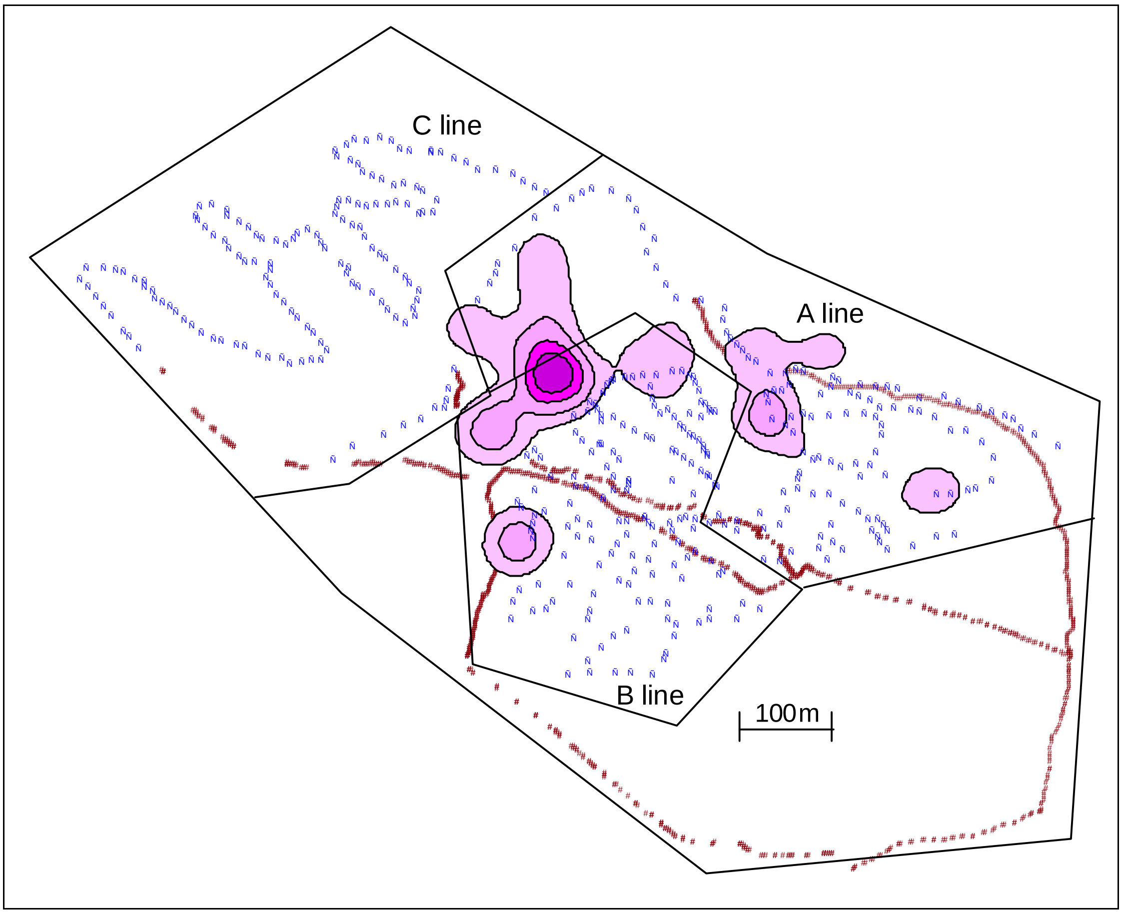
Appendix 1.9

Kernel density estimate of den sites for radio tracked tuberculous possums. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000



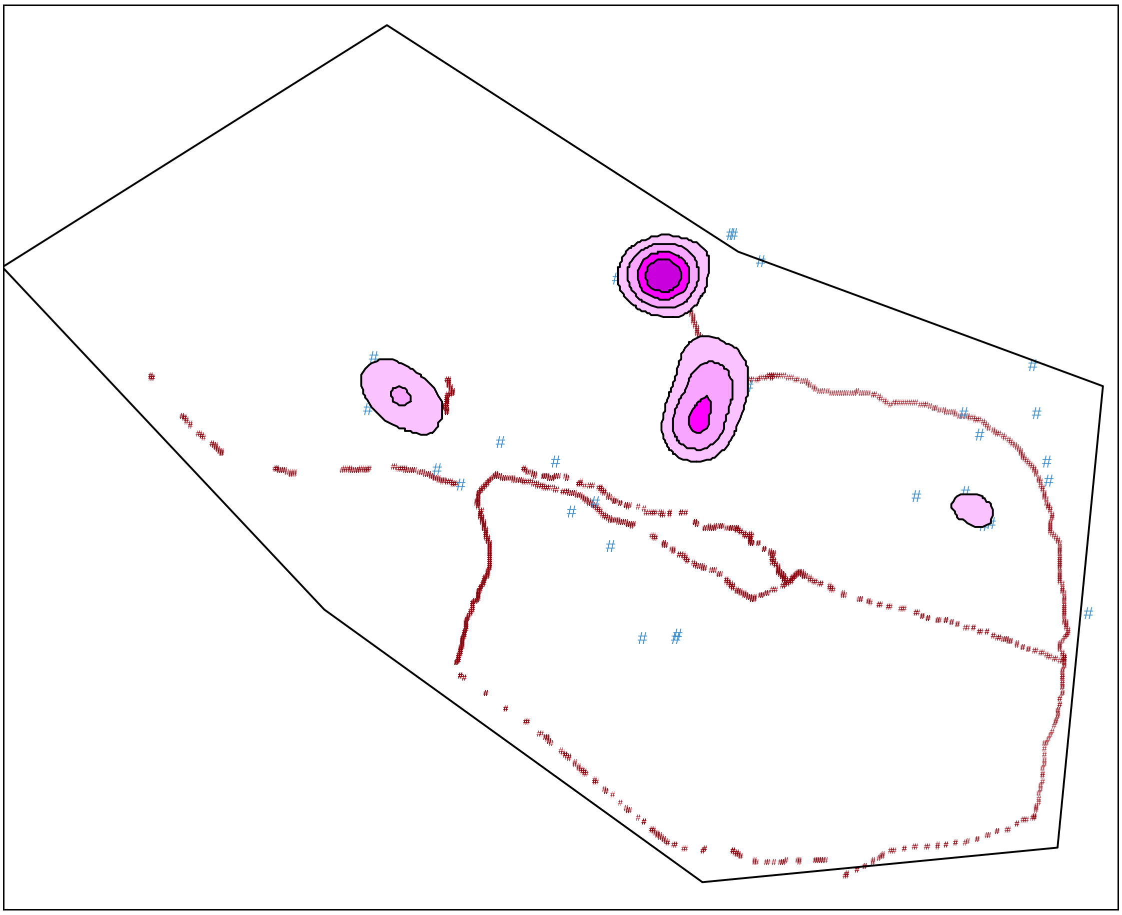
Appendix 1.10

Kernel density estimate of den sites for radio tracked naturally tuberculous possums. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000



Appendix 1.11

Kernel density estimate of den sites for experimentally infected tuberculous possums. The thin line marks the perimeter of the study site, the thick line represents land marks such as fence lines and tracks. Scale: 1:11000



APPENDIX II

The activity range, total range and summary statistics for radio tracked possums

This appendix includes summary information for individual possums, including activity and total range sizes, the number of long distance movements and cause of death. A graphic showing trap sites, den sites, activity range and total range is also included. Contours represent density of possum locations (a combination of den sites and trap sites), from high (central most 20% of den sites, darkest shade), through 40%, 60%, to low (80% of den sites, lightest shade).

Scale is 1:6000 for all graphics. Crosses represent trap sites where a possum was caught, dot points represent den sites.

Appendix 2.1

Possum ID number: 0001

Disease status: naturally infected

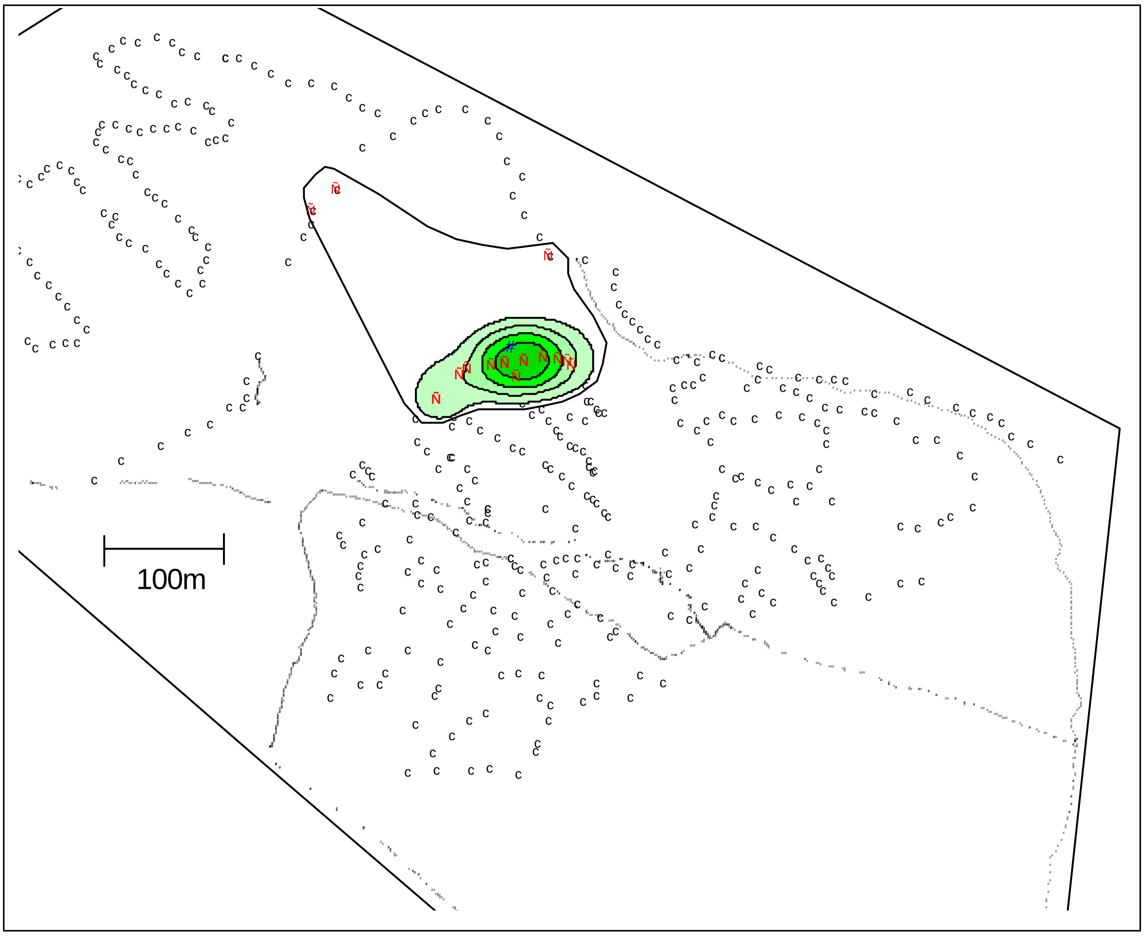
Possum sex: female

Activity range: 1.5ha

Total range: 5.5ha

Long distance movements: 2 (250m and 250m)

Cause of death: tuberculosis, carcass collected for *post-mortem* examination.



Appendix 2.2

Possum ID number: 0025

Disease status: experimentally infected (group two)

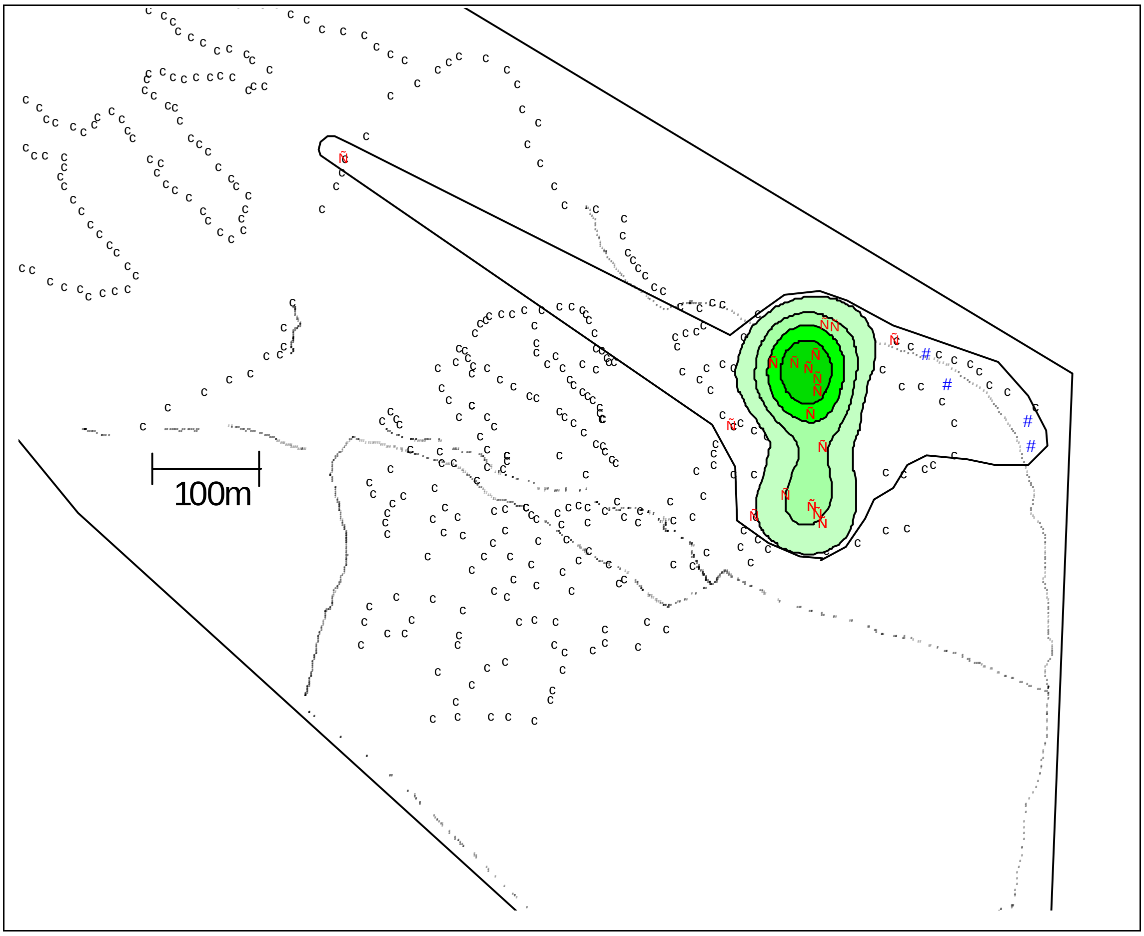
Possum sex: male

Activity range: 3.8ha

Total range: 10.6ha

Long distance movements: 1 (490m)

Cause of death: tuberculosis, carcass collected for *post-mortem* examination.



Appendix 2.3

Possum ID number: 0026

Disease status: control

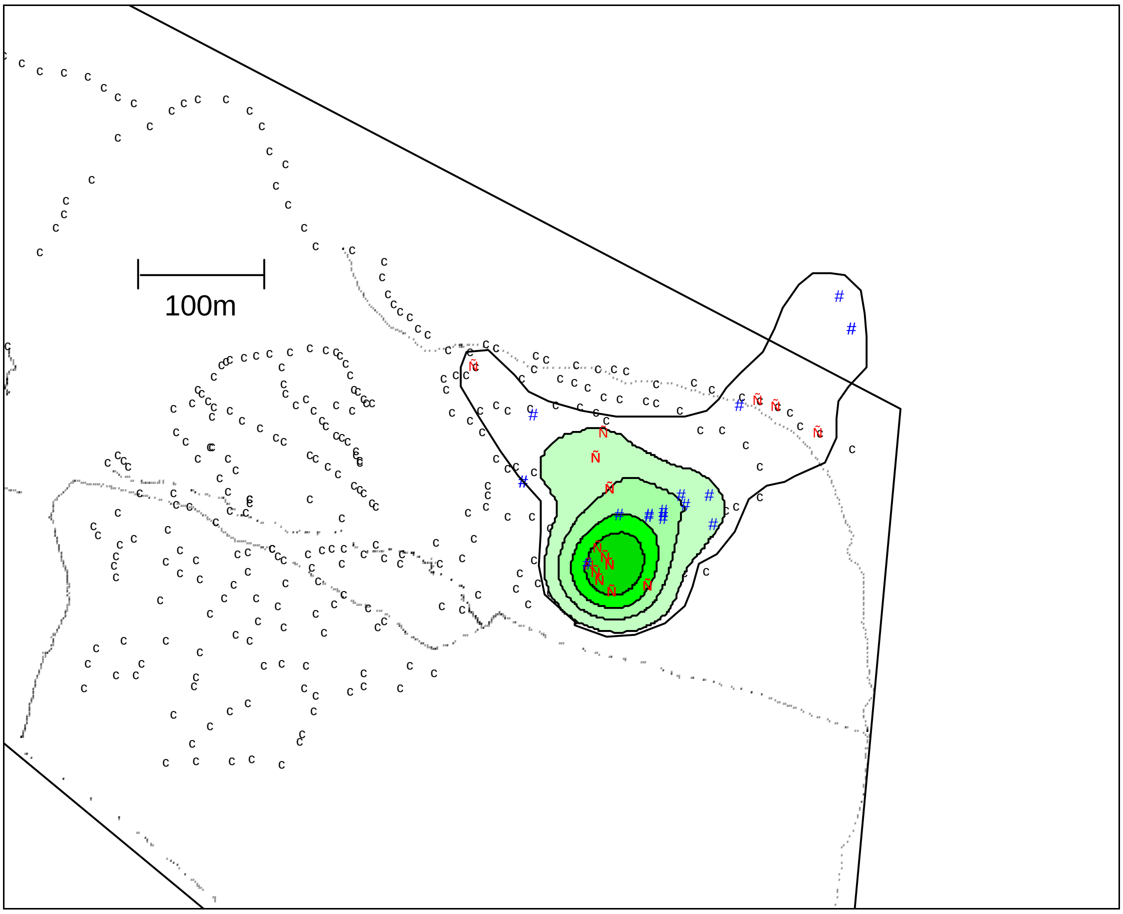
Possum sex: male

Activity range: 3.2ha

Total range: 7.6ha

Long distance movements: 3, (240m, 310m and 330m)

Cause of death: killed by the neighbouring farmer, carcass returned for *post-mortem* examination.



Appendix 2.4

Possum ID number: 0054

Disease status: naturally infected

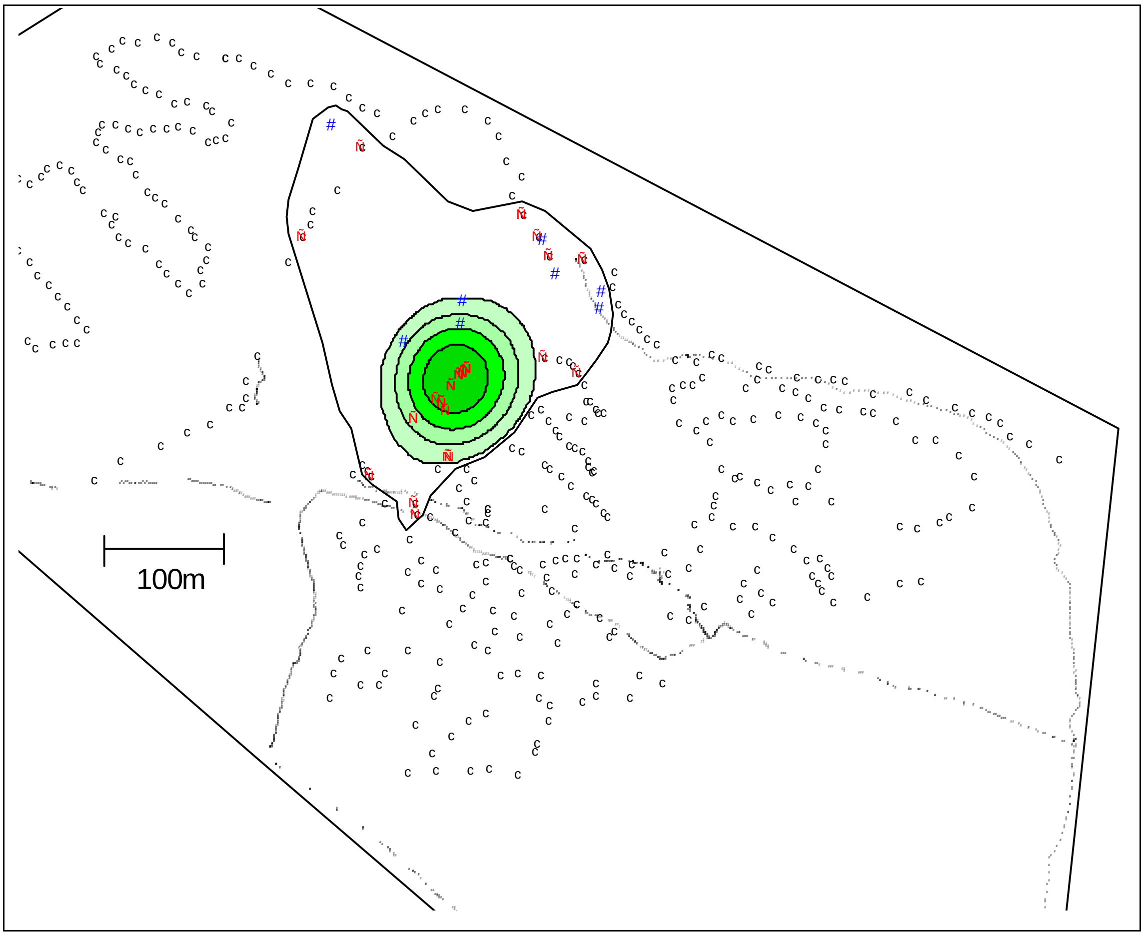
Possum sex: male

Activity range: 2.5ha

Total range: 9.5ha

Long distance movements: 3 (210m, 380m, 400m)

Cause of death: trapped by neighbouring farmer, carcass discarded.



Appendix 2.5

Possum ID number: 0055

Disease status: experimentally infected (group one)

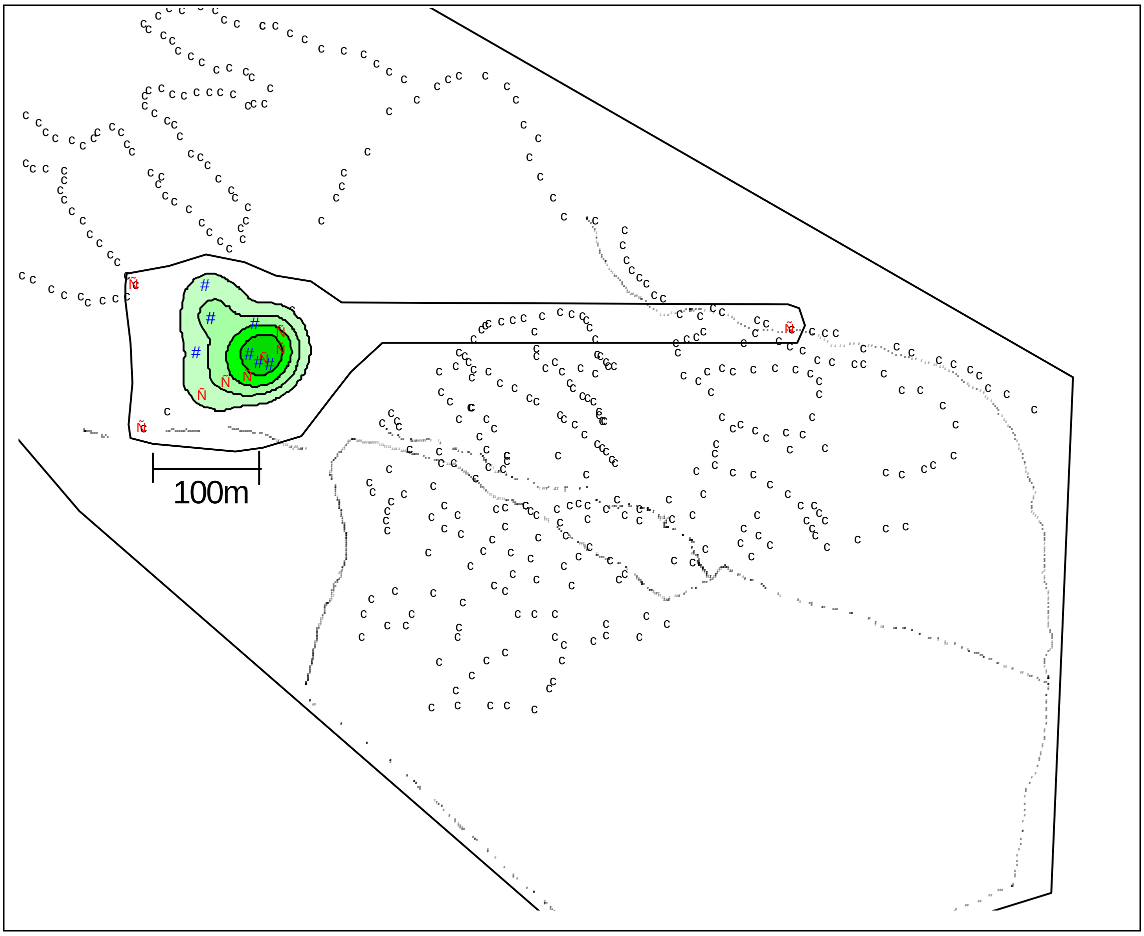
Possum sex: male

Activity range: 1.9ha

Total range: 7.6ha

Long distance movements: 1 (480m)

Cause of death: possum died during heart bleeding, carcass examined *post-mortem*.



Appendix 2.6

Possum ID number: 0084

Disease status: naturally infected

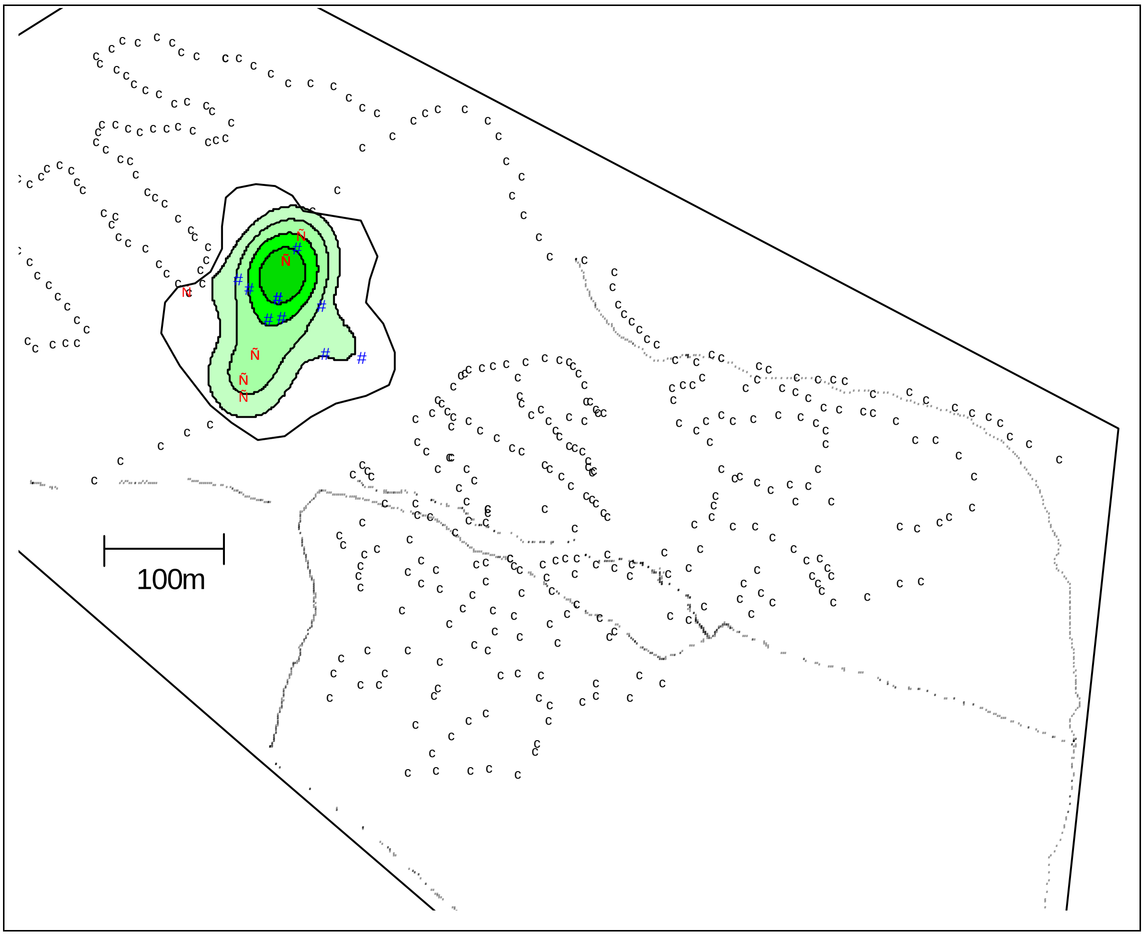
Possum sex: female

Activity range: 2.3ha

Total range: 4.9ha

Long distance movements: 0

Cause of death: tuberculosis, carcass collected for *post-mortem* examination.



Appendix 2.7

Possum ID number: 0111

Disease status: naturally infected

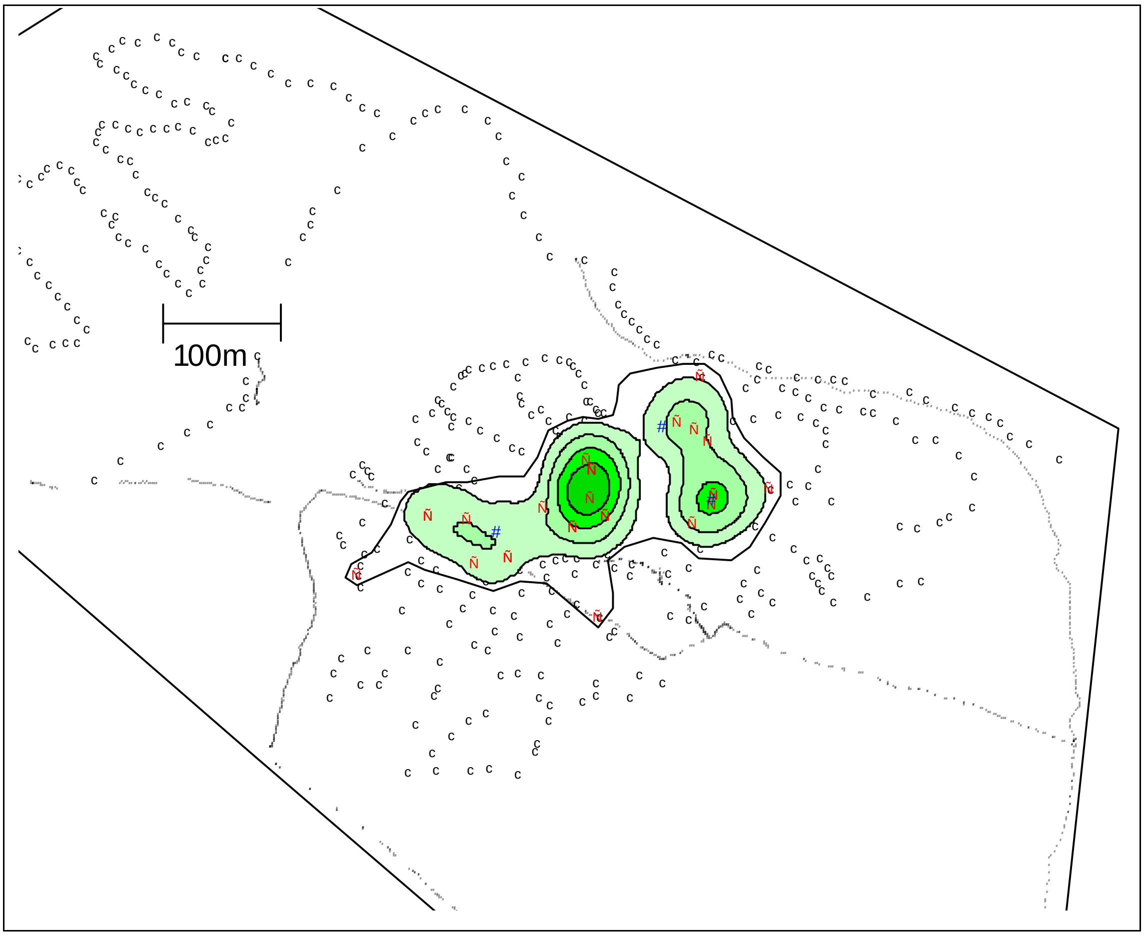
Possum sex: male

Activity range: 4.4ha

Total range: 6.7ha

Long distance movements: 1, (220m)

Cause of death: tuberculosis, carcass collected for *post-mortem* examination.



Appendix 2.8

Possum ID number: 0142

Disease status: control

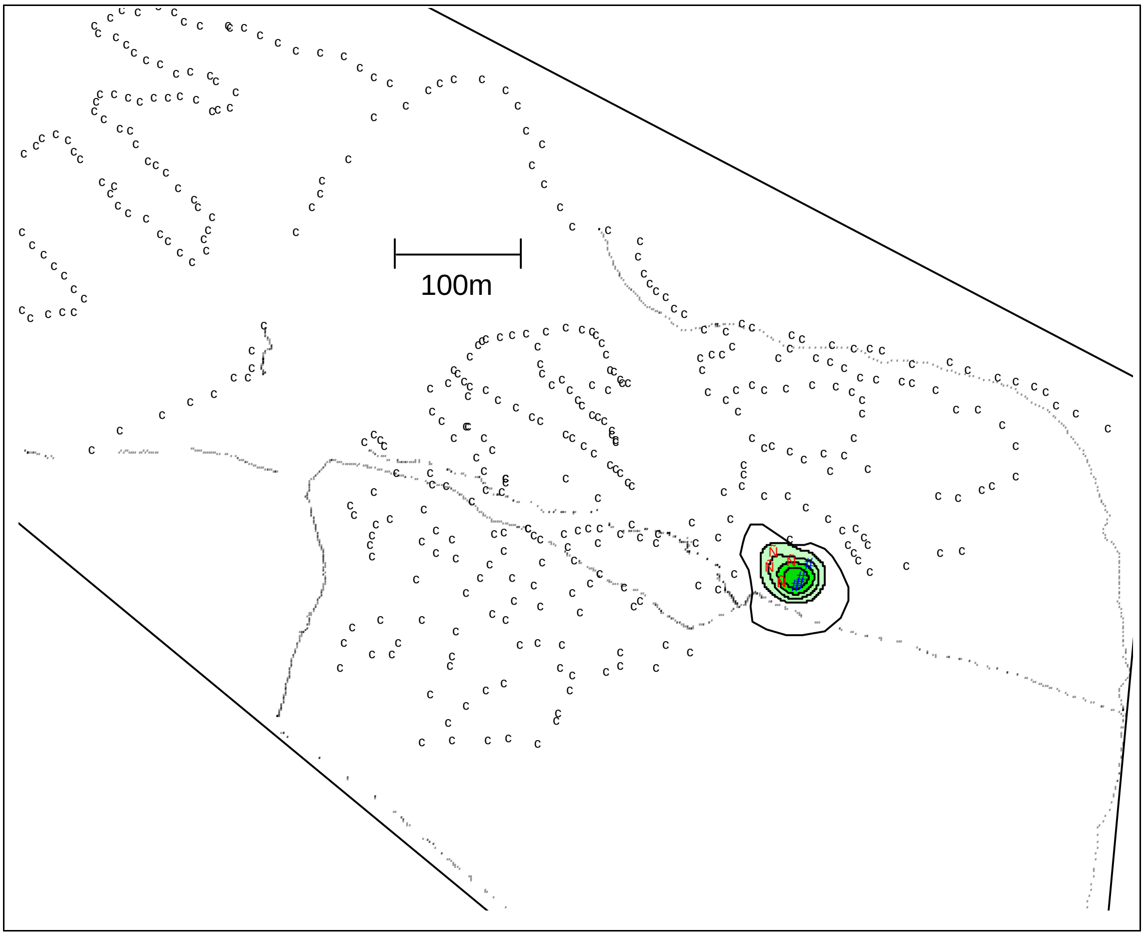
Possum sex: male

Activity range: 0.4ha

Total range: 1.0ha

Long distance movements: 0

Cause of death: trapped by neighbouring farmer, carcass returned for *post-mortem* examination.



Appendix 2.9

Possum ID number: 0151

Disease status: experimentally infected (group two)

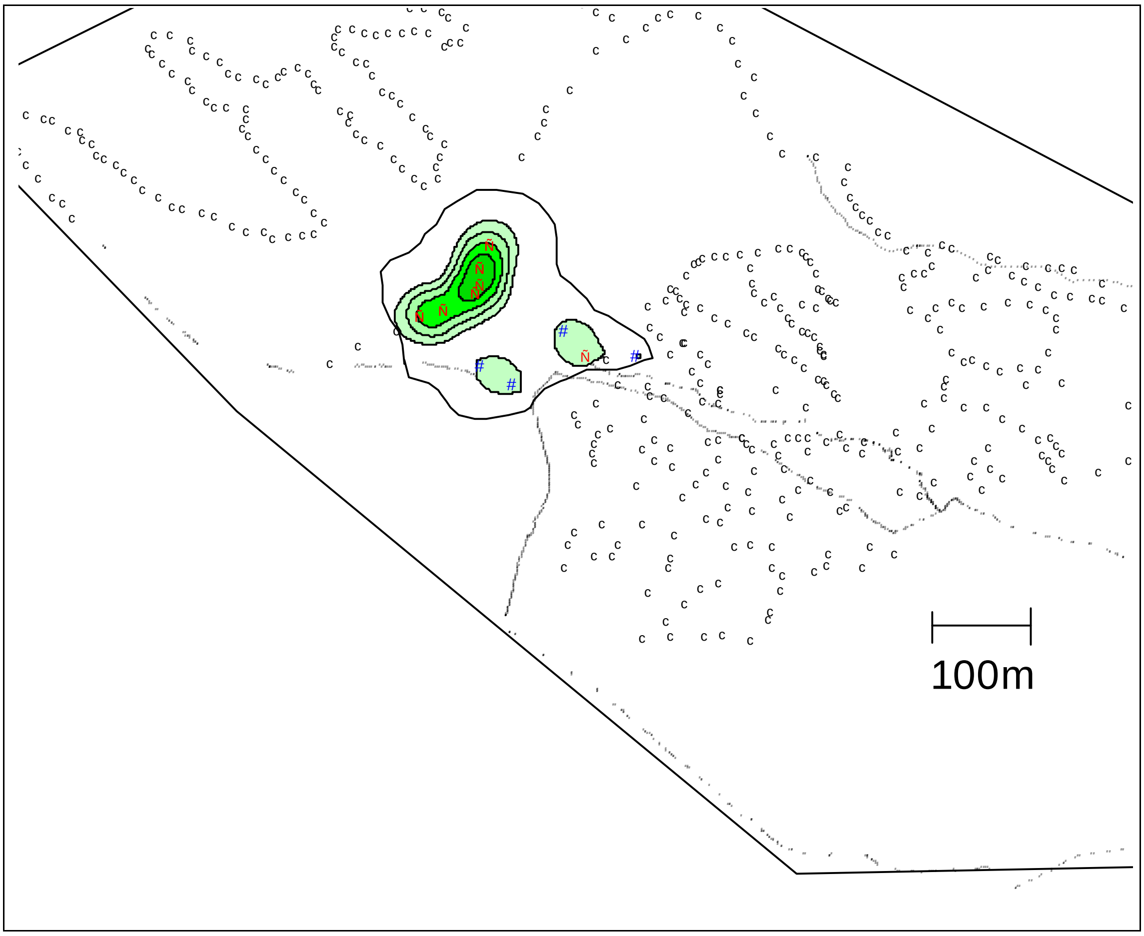
Possum sex: male

Activity range: 1.6ha

Total range: 4.8ha

Long distance movements: 0

Cause of death: tuberculosis, carcass collected for *post-mortem* examination.



Appendix 2.10

Possum ID number: 0159

Disease status: control

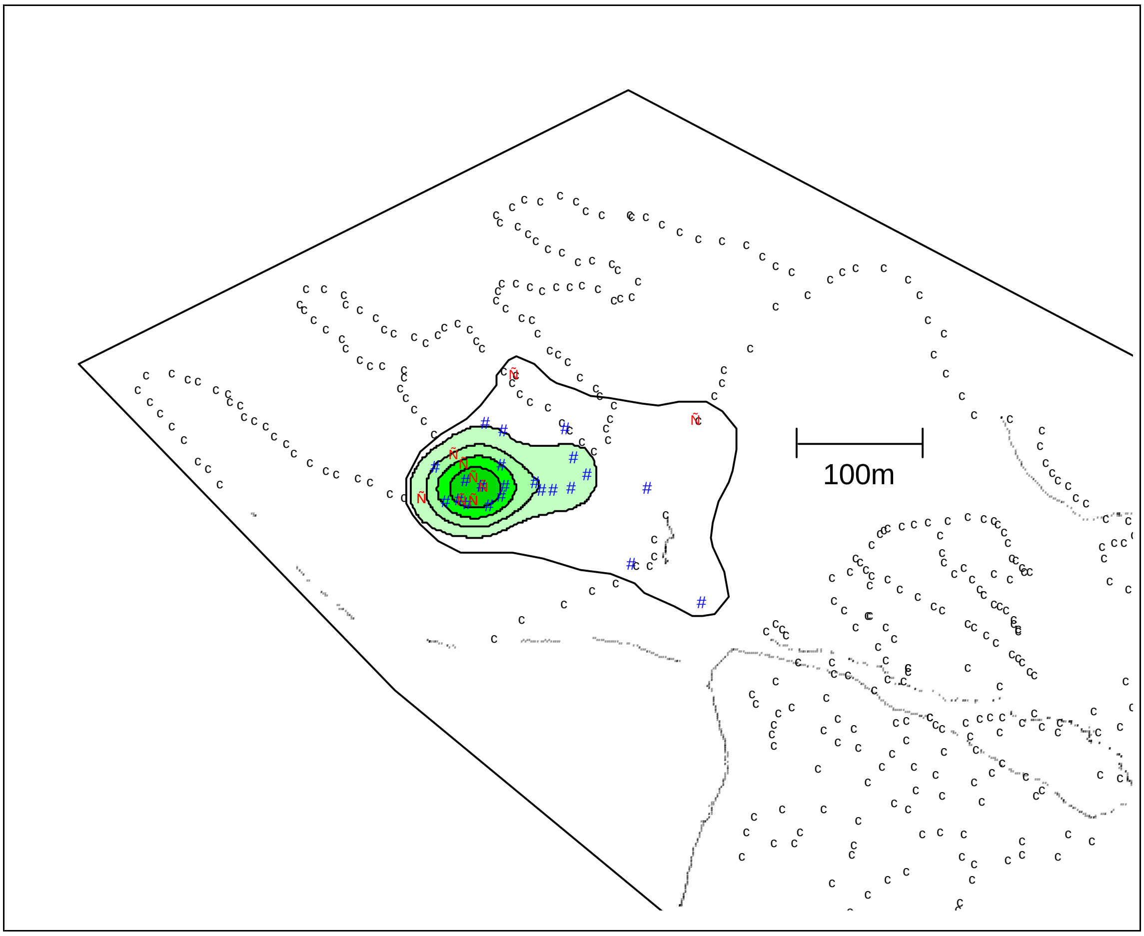
Possum sex: female

Activity range: 1.9ha

Total range: 6.3ha

Long distance movements: 1 (220m)

Cause of death: euthanasia during final depopulation of the study site, carcass collected for *post-mortem* examination.



Appendix 2.11

Possum ID number: 0164

Disease status: control

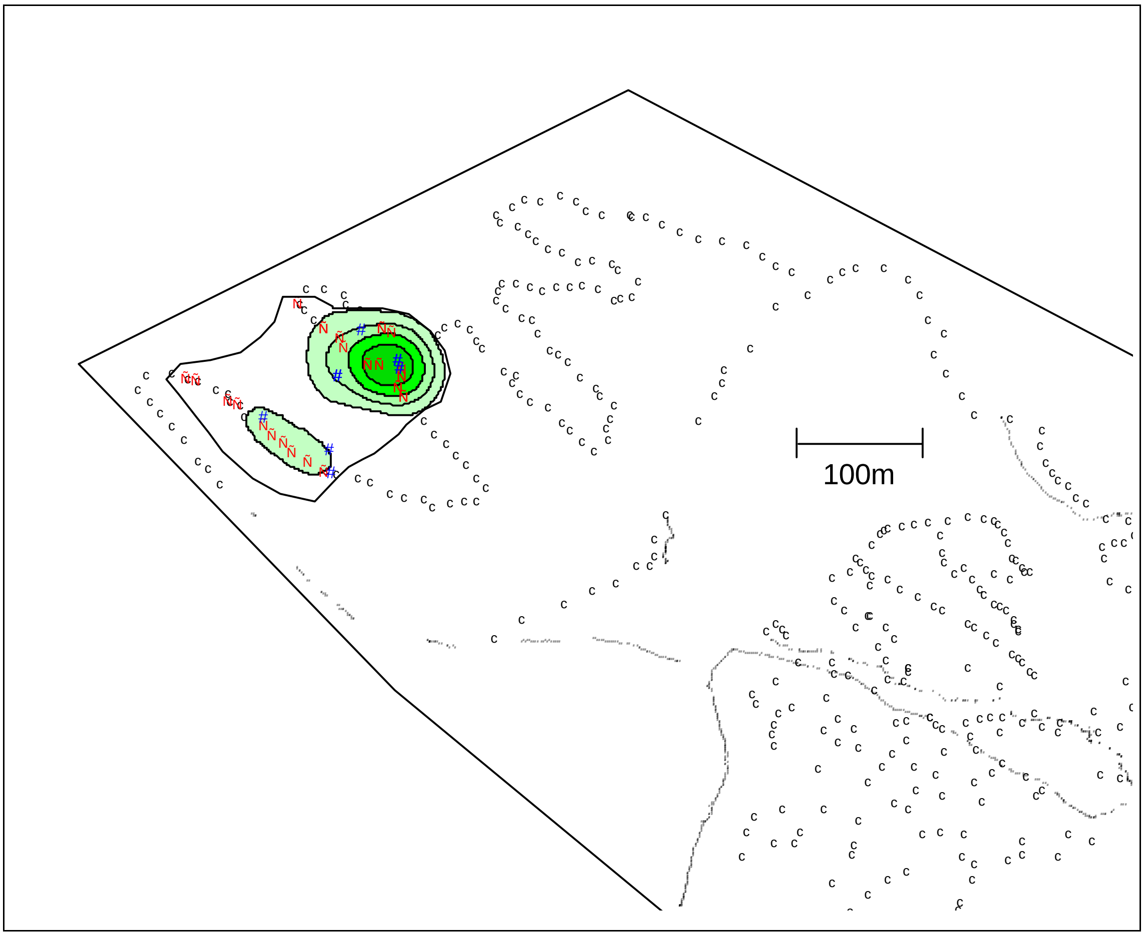
Possum sex: female

Activity range: 1.7ha

Total range: 4.1ha

Long distance movements: 0

Cause of death: radio collar was removed after it caused an abscess to form on the neck of the possum. This possum was euthanased during final depopulation of study site and the carcass examined *post-mortem*.



Appendix 2.12

Possum ID number: 0183

Disease status: control

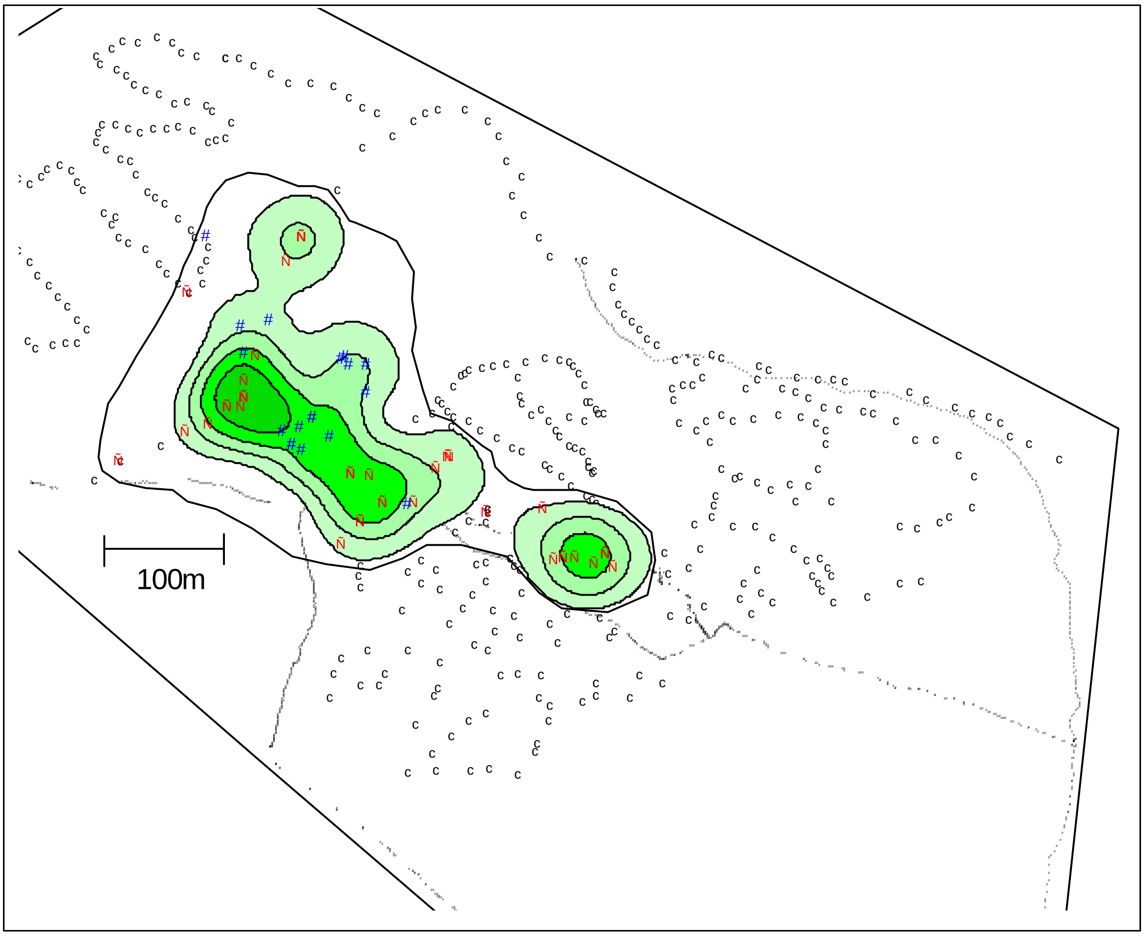
Possum sex: male

Activity range: 6.1ha

Total range: 13.6ha

Long distance movements: 0

Cause of death: euthanasia during final depopulation of the study site, carcass examined *post-mortem*.



Appendix 2.13

Possum ID number: 0209

Disease status: control

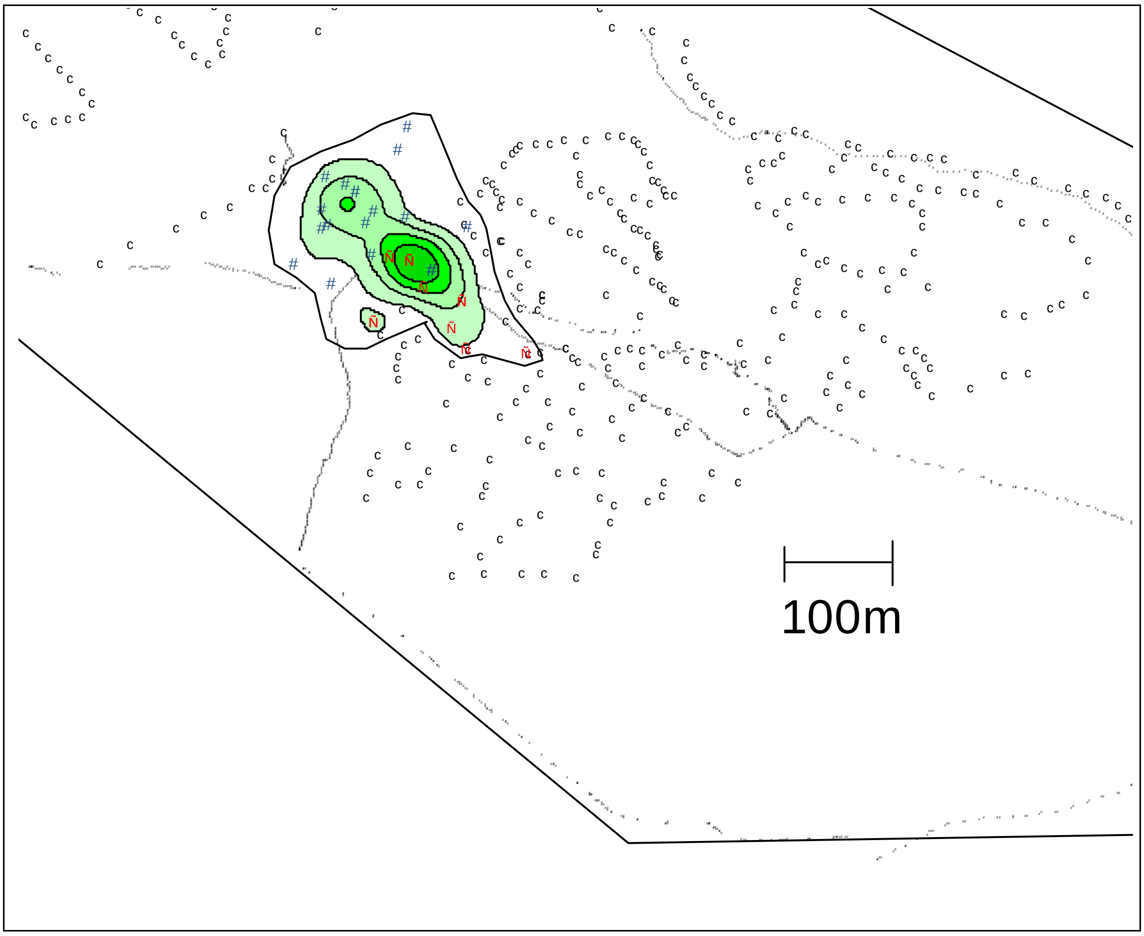
Possum sex: male

Activity range: 2.0ha

Total range: 3.9ha

Long distance movements: 0

Cause of death: euthanasia during final depopulation of the study site, carcass examined *post-mortem*.



Appendix 2.14

Possum ID number: 0283

Disease status: control

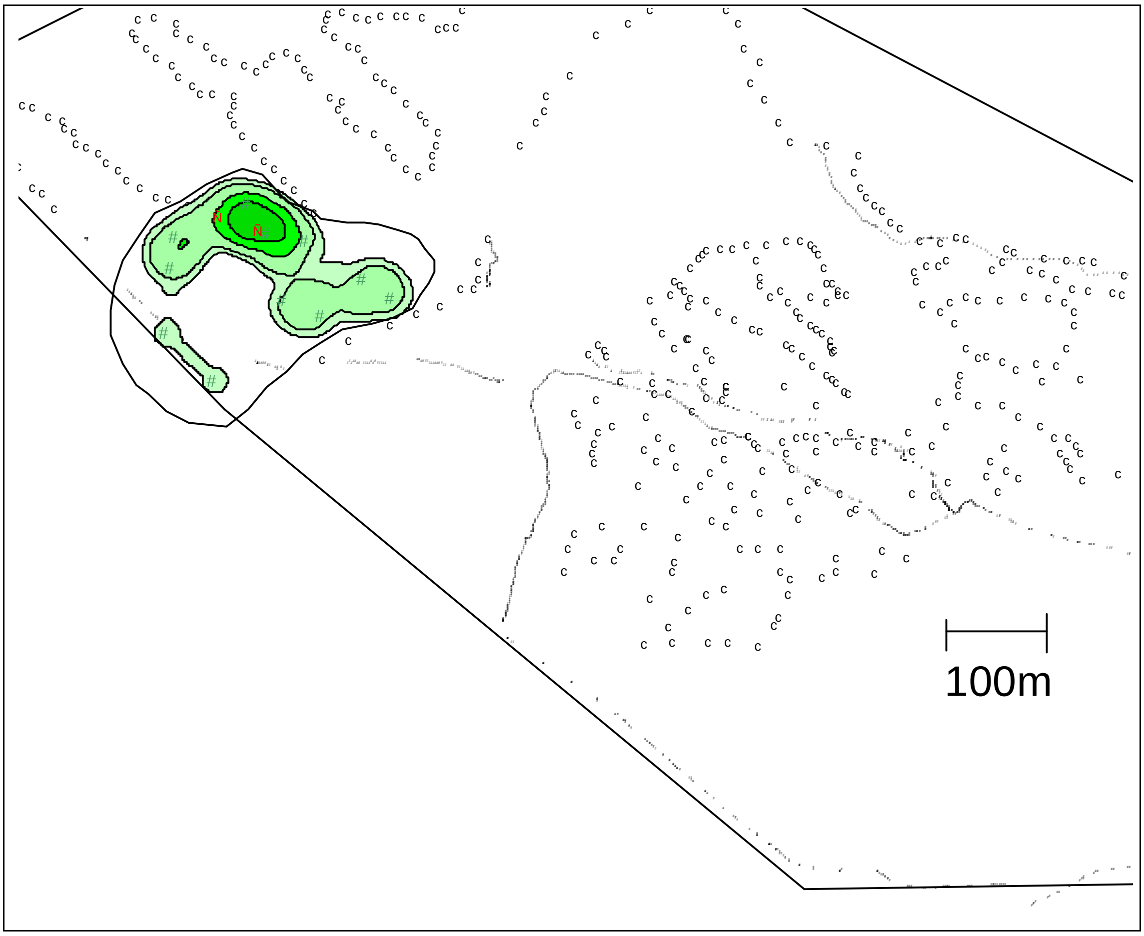
Possum sex: female

Activity range: 2.7ha

Total range: 6.1ha

Long distance movements: 0

Cause of death: euthanasia during final depopulation of the study site, carcass examined *post-mortem*.



Appendix 2.15

Possum ID number: 5625

Disease status: naturally infected

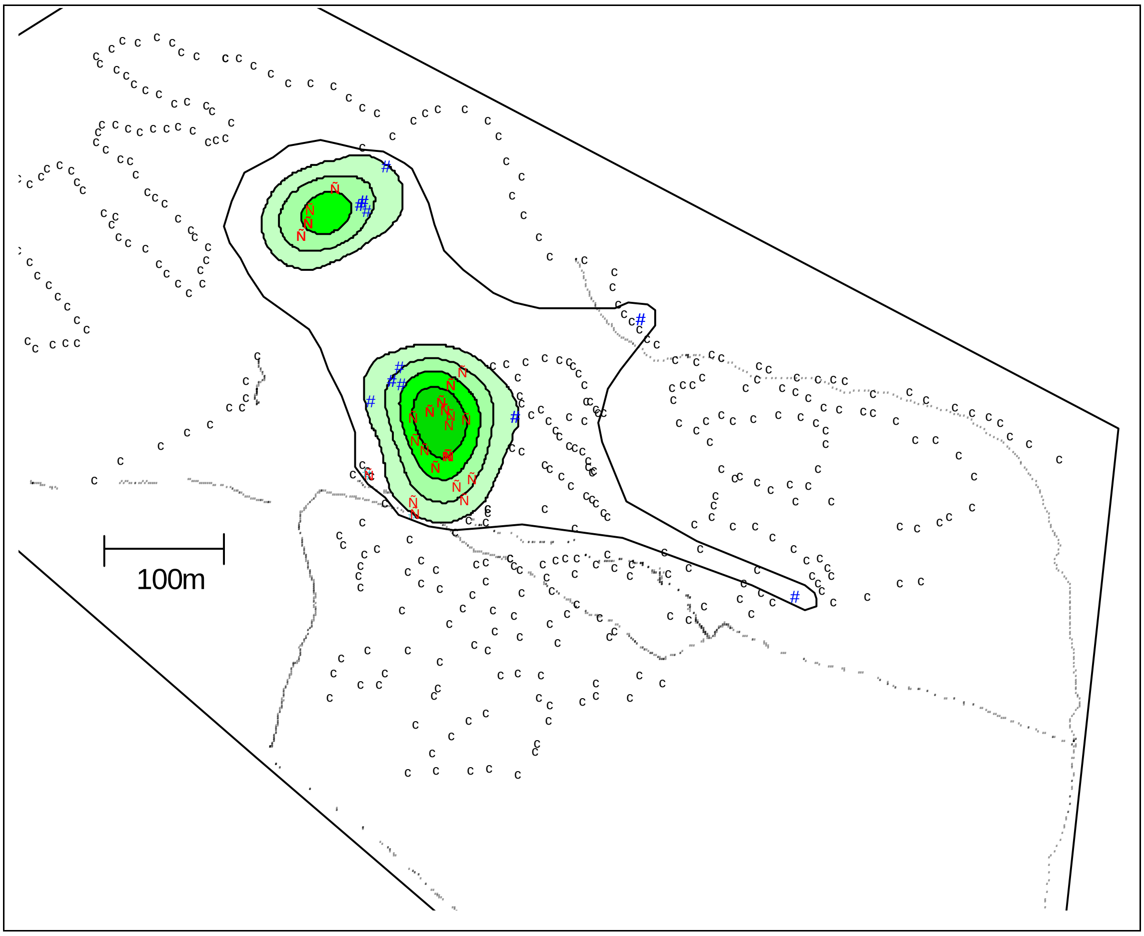
Possum sex: female

Activity range: 4.1ha

Total range: 13.4ha

Long distance movements: 1 (350m)

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.16

Possum ID number: 5644

Disease status: naturally infected

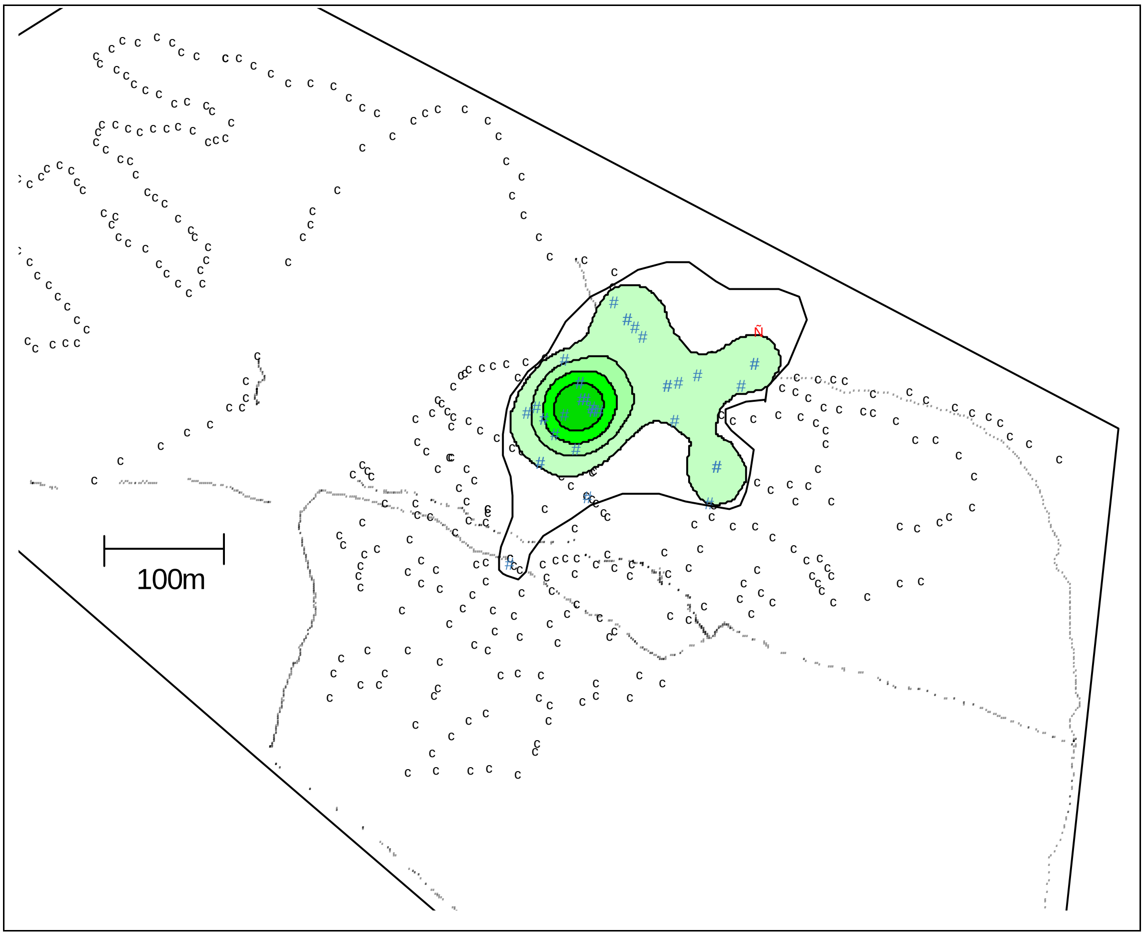
Possum sex: male

Activity range: 2.9ha

Total range: 6.5ha

Long distance movements: 0

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.17

Possum ID number: 5697

Disease status: control

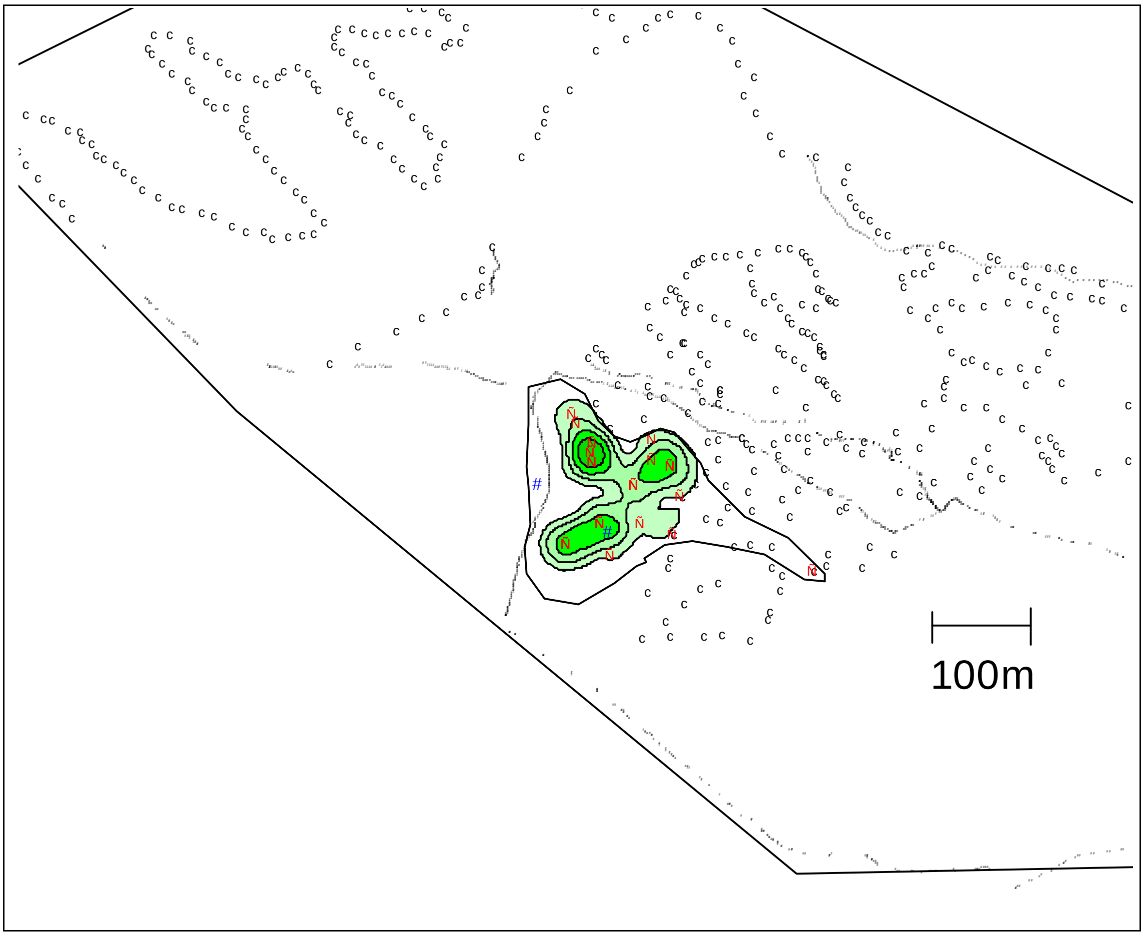
Possum sex: male

Activity range: 2.1ha

Total range: 4.2ha

Long distance movements: 1 (225m)

Cause of death: destroyed by bulldozer clearing scrub.



Appendix 2.18

Possum ID number: 5700

Disease status: naturally infected

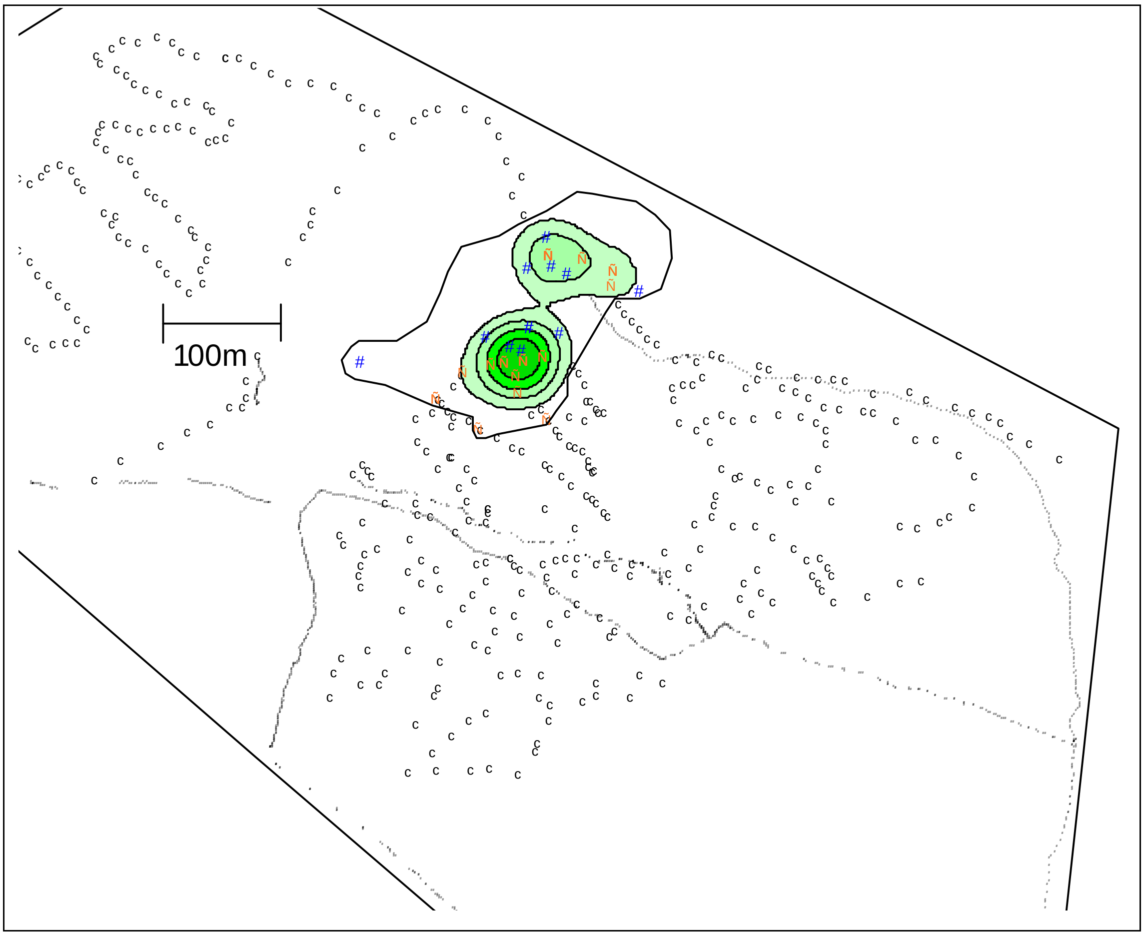
Possum sex: male

Activity range: 2.2ha

Total range: 5.1ha

Long distance movements: 0

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.19

Possum ID number: 5758

Disease status: experimentally infected (group one)

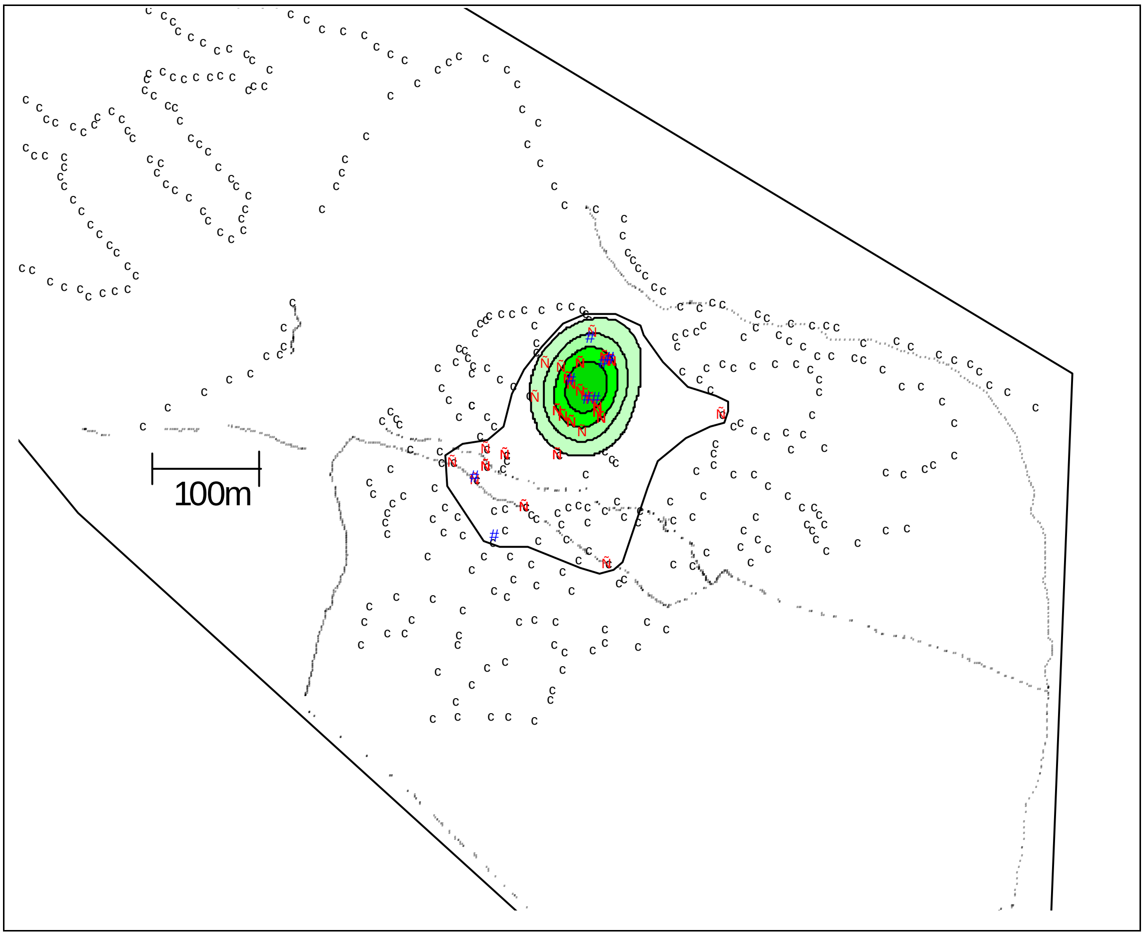
Possum sex: male

Activity range: 1.7ha

Total range: 5.5ha

Long distance movements: 0

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.20

Possum ID number: 5760

Disease status: experimentally infected (group one)

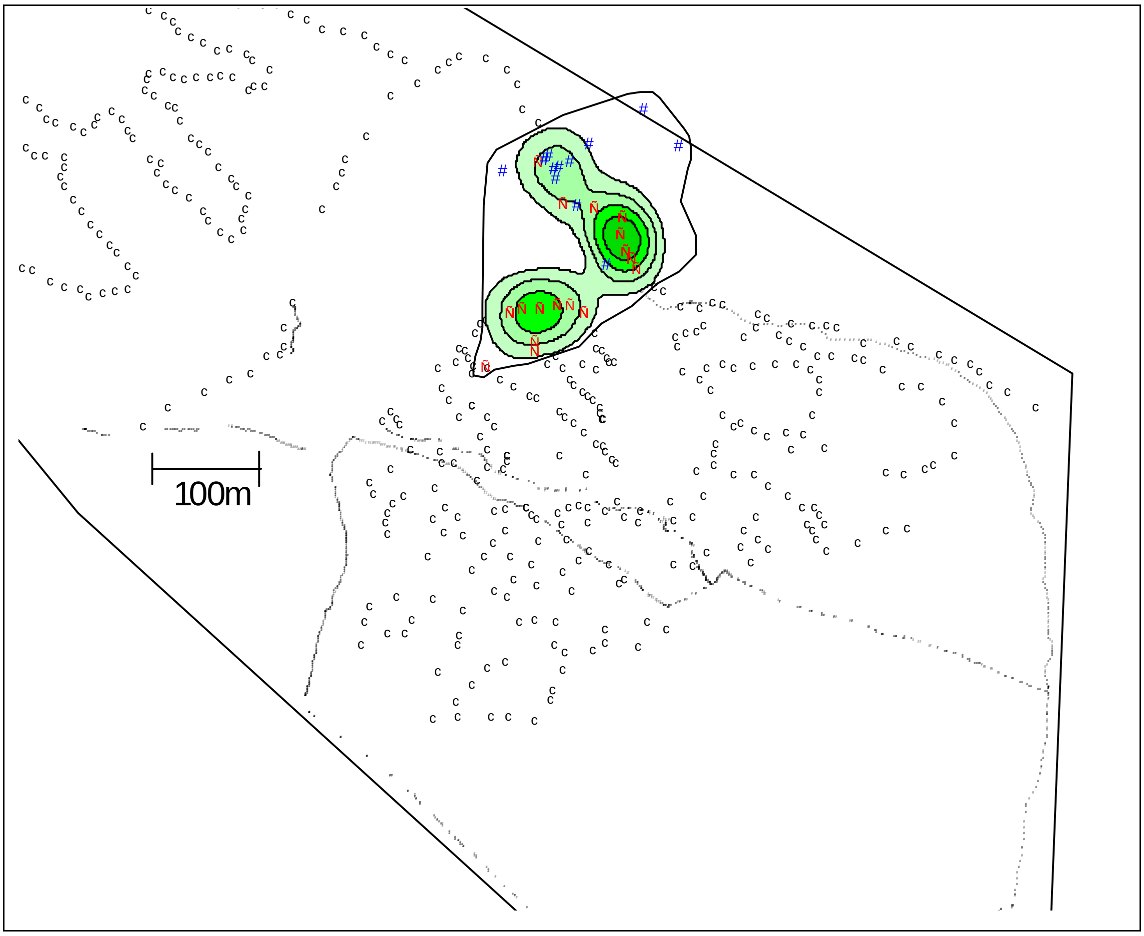
Possum sex: male

Activity range: 3.0ha

Total range: 5.9ha

Long distance movements: 0

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.21

Possum ID number: 5768

Disease status: naturally infected

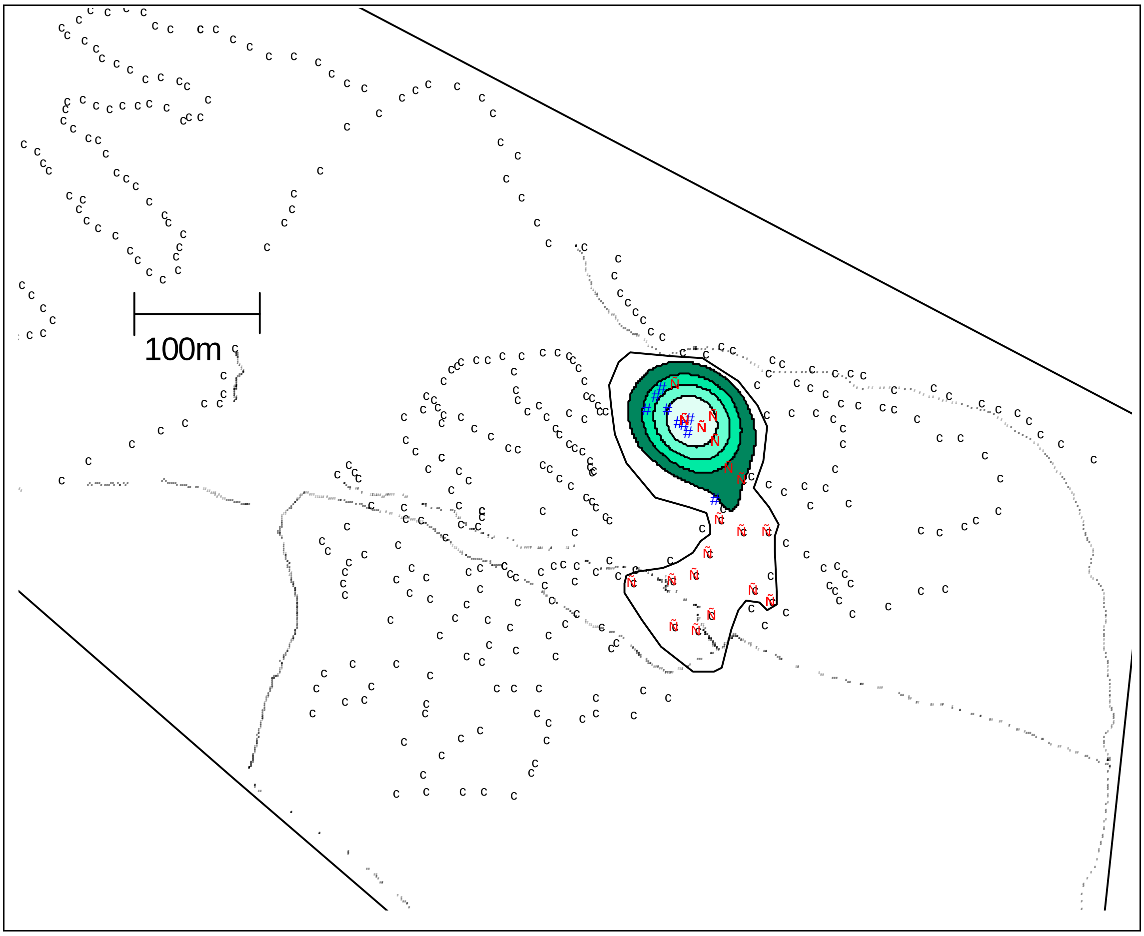
Possum sex: female

Activity range: 1.5ha

Total range: 3.7ha

Long distance movements: 0

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.22

Possum ID number: 5787

Disease status: naturally infected

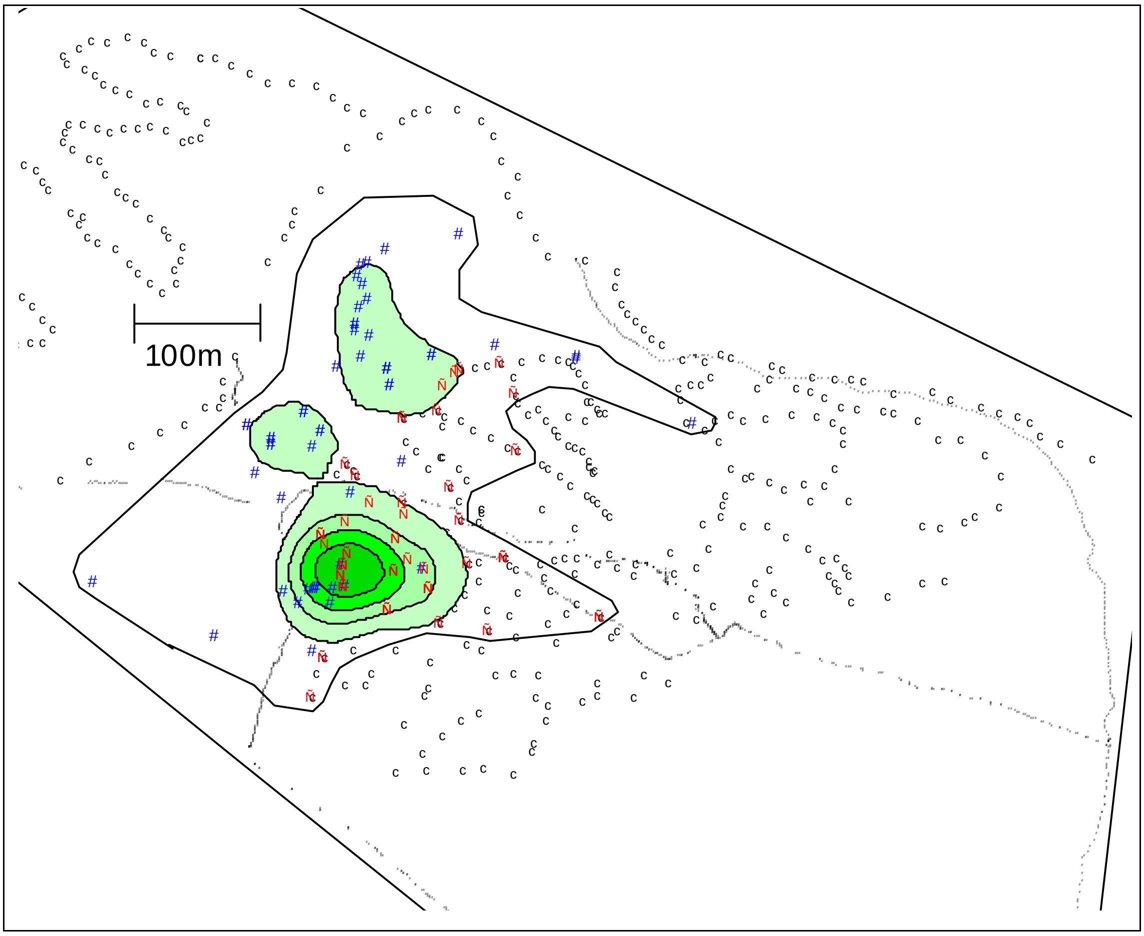
Possum sex: male

Activity range: 4.6ha

Total range: 17.0ha

Long distance movements: 3, (210m, 210m and 320m)

Cause of death: euthanasia during final depopulation of the study site, carcass examined *post-mortem*.



Appendix 2.23

Possum ID number: 5796

Disease status: experimentally infected (group one)

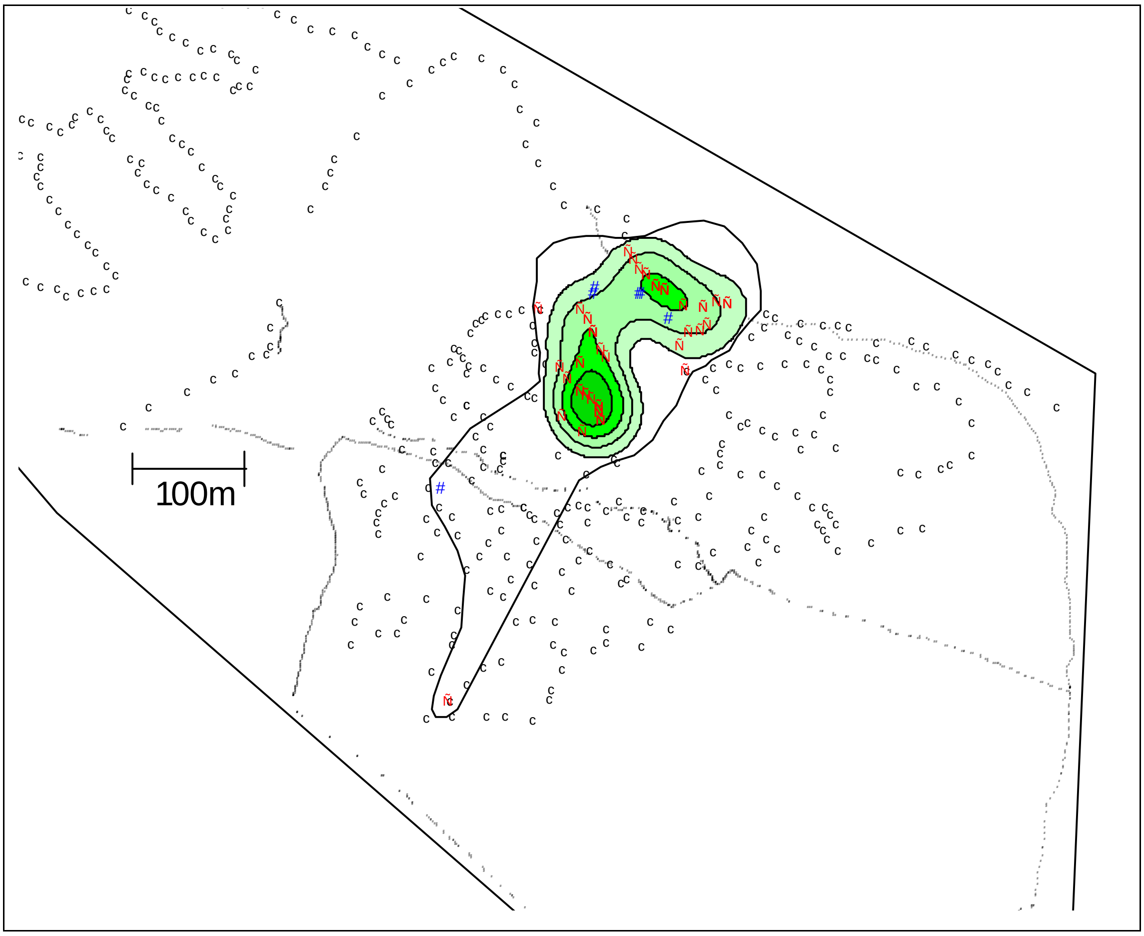
Possum sex: male

Activity range: 3.0ha

Total range: 8.7ha

Long distance movements: 1 (350m)

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.24

Possum ID number: 5816

Disease status: naturally infected

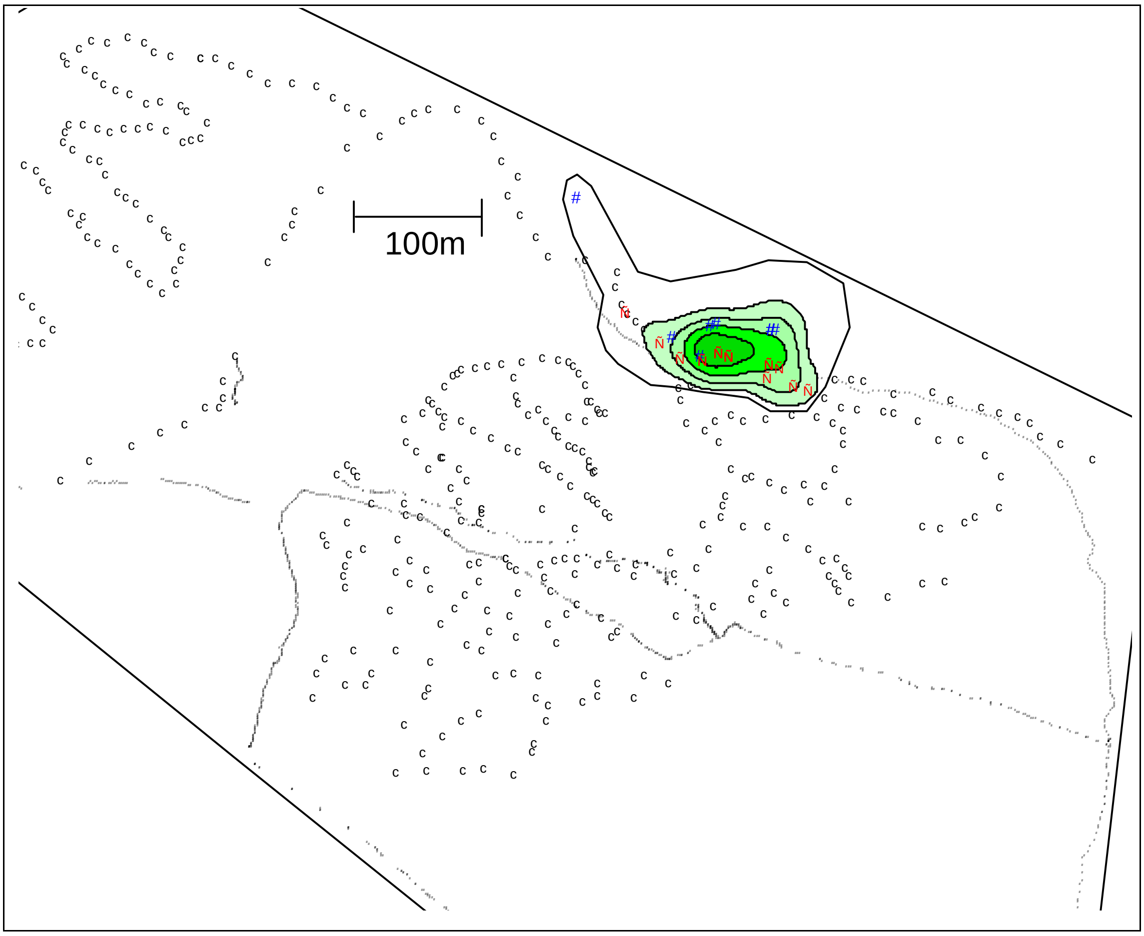
Possum sex: female

Activity range: 1.6ha

Total range: 3.7ha

Long distance movements: 1, (210m)

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.25

Possum ID number: 5831

Disease status: naturally infected

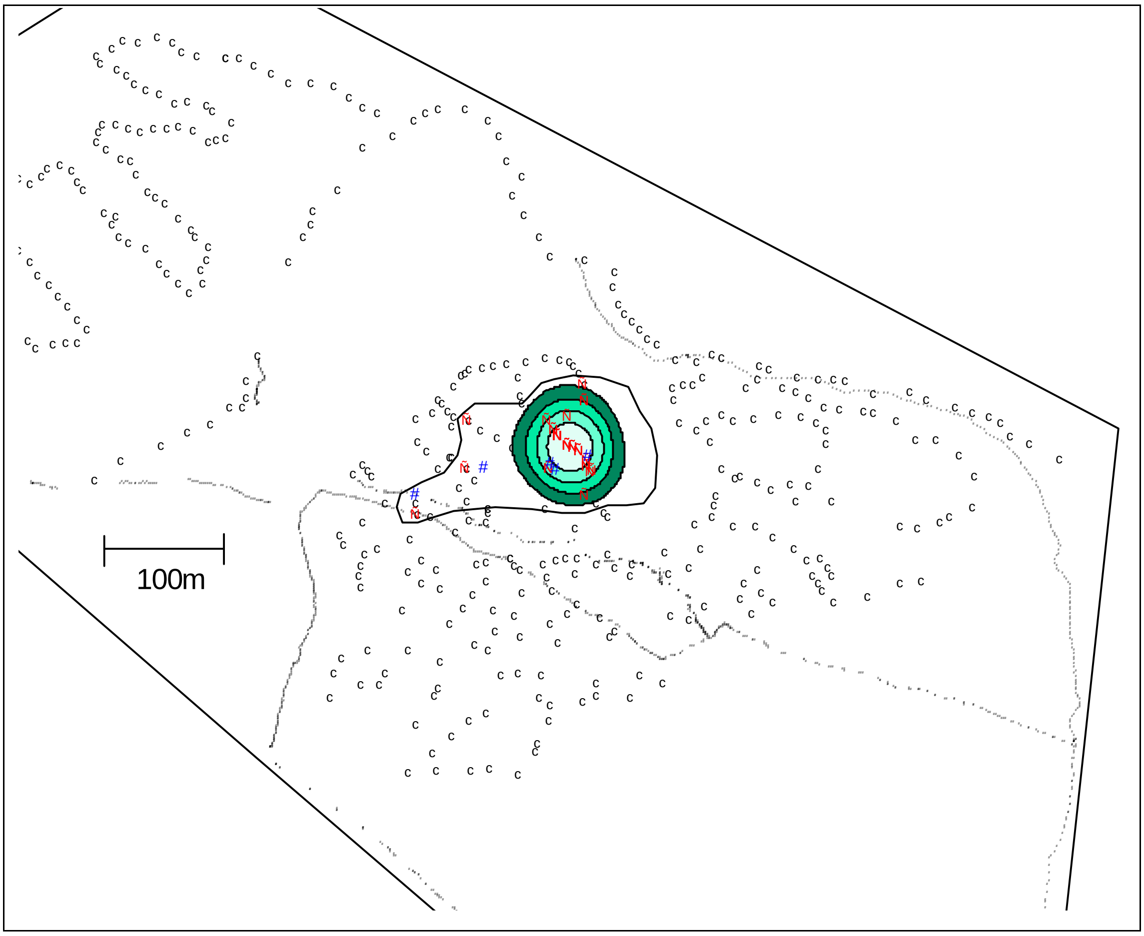
Possum sex: female

Activity range: 1.4ha

Total range: 3.0ha

Long distance movements: 0

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.26

Possum ID number: 5838

Disease status: experimentally infected (group 3)

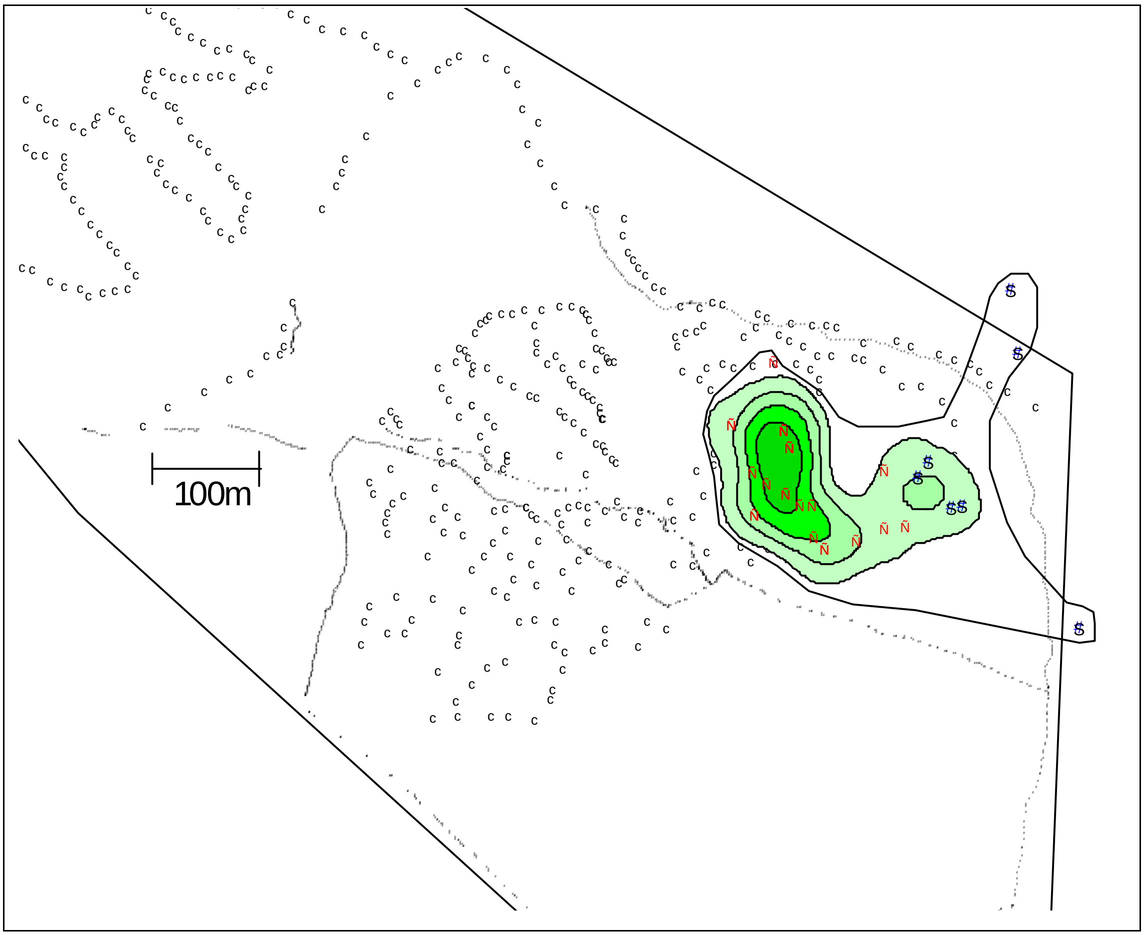
Possum sex: male

Activity range: 4.5ha

Total range: 8.9ha

Long distance movements: 3, (260m, 290m, 320m)

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.27

Possum ID number: 5871

Disease status: naturally infected

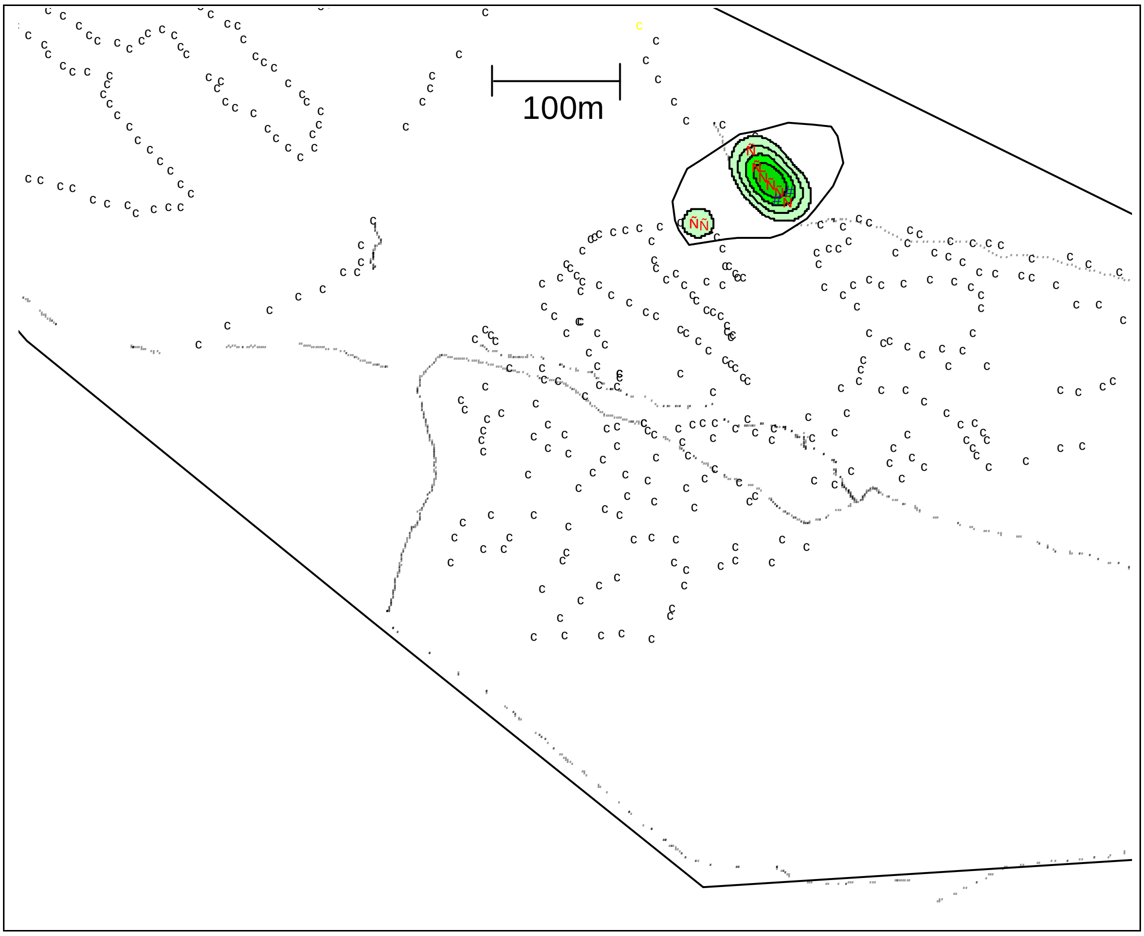
Possum sex: female

Activity range: 0.7ha

Total range: 1.7ha

Long distance movements: 0

Cause of death: tuberculosis, carcass examined *post-mortem*.



Appendix 2.28

Possum ID number: 5885

Disease status: experimentally infected (group two)

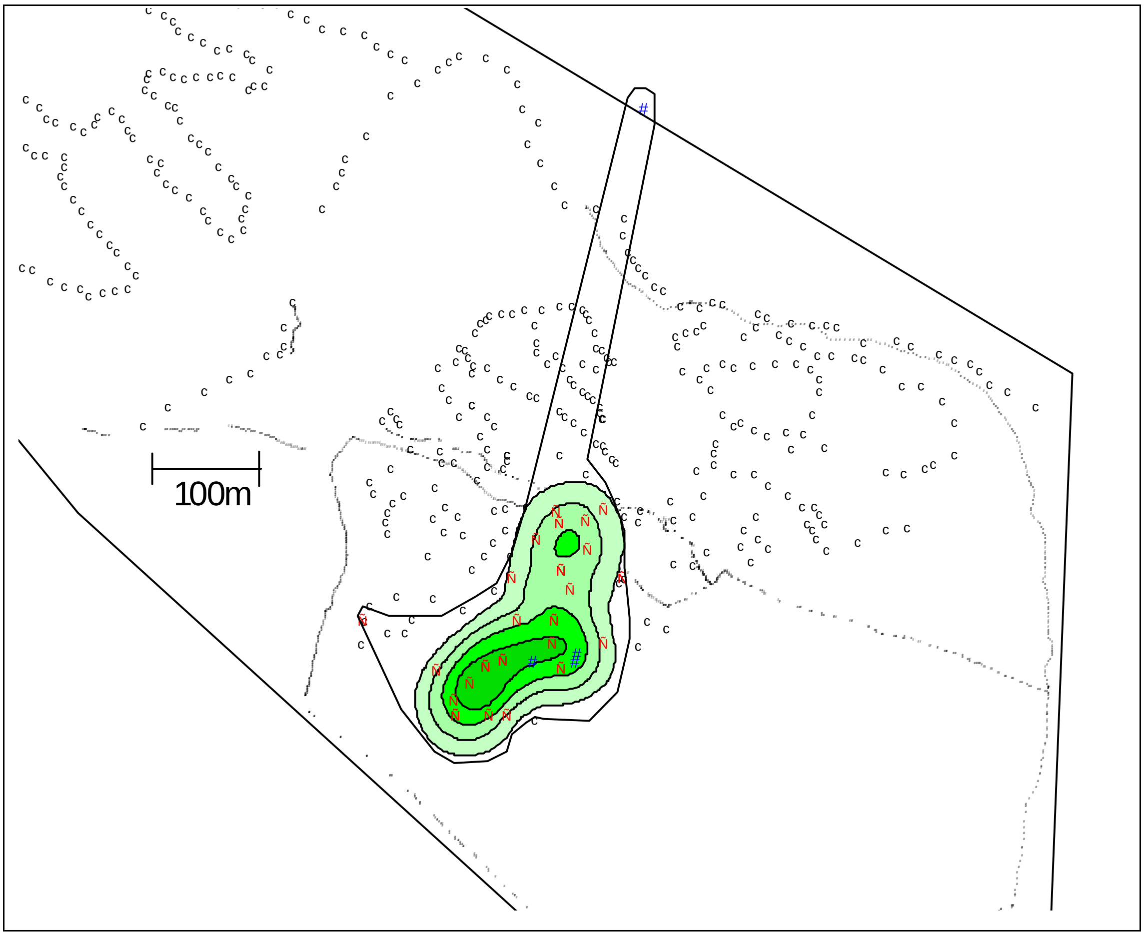
Possum sex: male

Activity range: 4.5ha

Total range: 8.0ha

Long distance movements: 1, (660m)

Cause of death: unknown, possum lost due to radio transmitter failure.



APPENDIX III

Radio tracking form

RADIO TRACKING FORM

Tracker ID date collar

Ear tag TB status seen/flushed

Dist to trap trap no. bearing den height roof material

Floor material floor condition no. of entrances size

Denface slope bearing of slope wind proneness surroundings

Weather today last night yesterday

comment

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